ASSESSING TIME SINCE DEATH BY USING CHANGES IN ELECTROLYTES IN C.S.F AND VITREOUS HUMOUR IN BODIES SUBJECTED TO AUTOPSY

Dissertation submitted for partial fulfillment of the requirement for the Degree of M.D (FORENSIC MEDICINE) BRANCH – XIV

DEPARTMENT OF FORENSIC MEDICINE

TIRUNELVELI MEDICAL COLLEGE

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BONAFIDE CERTIFICATE

This is to certify that the work in this dissertation entitled "**ASSESSING TIME SINCE DEATH BY USING CHANGES IN ELECTROLYTES IN CSF AND VITREOUS HUMOUR IN BODIES SUBJECTED TO AUTOPSY**" - has been carried out by **Dr.Rama.V M.B.BS, D.M.R.T,** a post graduate student under my supervision and guidance for her study leading to Branch XIV M.D Degree in Forensic Medicine during the period of June 2015 – May 2018.

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DECLARATION

I, Dr. Rama.V M.B.B.S, D.M.R.T, solemnly declare that this dissertation titled "**ASSESSING TIME SINCE DEATH BY USING CHANGES IN ELECTROLYTES IN CSF AND VITREOUS HUMOUR IN BODIES SUBJECTED TO AUTOPSY**" is a bonafide work done by me , under the expert guidance and supervision of **Dr.A.Selvamurugan, M.D, DNB, MNAMS.,** Professor and Head of the Department of Forensic Medicine, Tirunelveli Medical College, Tirunelveli. This dissertation is submitted to The Tamil Nadu Dr. M.G.R. Medical University towards partial fulfillment for the award of M.D Degree (Branch XIV) in Forensic Medicine.

Place :

Date : Dr.Rama.V,

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I am extremely thankful to the past & present Deans of Tirunelveli Medical College, Tirunelveli, for granting me permission to do this study.

ABBREVATIONS:

&	–	And
<	–	Less than
>	–	Greater than
Ed	–	Edition
R	–	Right
L	–	Left
M	–	Male
F	–	Female
VH	–	Vitreous humour
CSF	–	Cerebrospinal fluid
i.e	–	That is
Sl.No	–	Serial Number
PM.no	–	Postmortem number
PMI	–	Post mortem interval
Yrs	–	Years
TSD	–	Time Since Death
COD	–	Cause of Death

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PROTOCOL TITLE: ASSESSING TIME SINCE DEATH BY USING CHANGES IN ELECTROLYTES IN CSF AND VITREOUS HUMOR IN BODIES SUBJECTED TO AUTOPSY

PRINCIPAL INVESTIGATOR: DR.V.RAMA, MBBS.,DMRT.,

DESIGNATION OF PRINCIPAL INVESTIGATOR POST GRADUATE IN FORENSIC MEDICINE
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Dear . Dr.V.Rama, MBBS.,DMRT., The Tirunelveli Medical College Institutional Ethics Committee (TIREC) reviewed and discussed your application during the IEC meeting held on 10.12.15.

THE FOLLOWING DOCUMENTS WERE REVIEWED AND APPROVED

1.	TIREC Application Form	☐
2.	Study Protocol	☐
3.	Department Research Committee Approval	☐
4.	Patient Information Document and Consent Form in English and Vernacular Language	☐
5.	Investigator's Brochure	☐
6.	Proposed Methods for Patient Accrual Proposed	☐
7.	Curriculum Vitae of the Principal Investigator	☐
8.	Insurance /Compensation Policy	☐
9.	Investigator's Agreement with Sponsor	☐
10.	Investigator's Undertaking	☐
11.	DCGI/DGFT approval	☐
12.	Clinical Trial Agreement (CTA)	☐
13.	Memorandum of Understanding (MOU)/Material Transfer Agreement (MTA)	☐
14.	Clinical Trials Registry-India (CTRI) Registration	☐

THE PROTOCOL IS APPROVED IN ITS PRESENTED FORM ON THE FOLLOWING CONDITIONS

1. The approval is valid for a period of 2 year/s or duration of project whichever is later
2. The date of commencement of study should be informed
3. A written request should be submitted 3weeks before for renewal / extension of the validity
4. An annual status report should be submitted.
5. The TIREC will monitor the study
6. At the time of PI's retirement/leaving the institute, the study responsibility should be transferred to a person cleared by HOD
7. The PI should report to TIREC within 7 days of the occurrence of the SAE. If the SAE is Death, the Bioethics Cell should receive the SAE reporting form within 24 hours of the occurrence.
8. In the events of any protocol amendments, TIREC must be informed and the amendments should be highlighted in clear terms as follows:
   a. The exact alteration/amendment should be specified and indicated where the amendment occurred in the original project. (Page no. Clause no. etc.)
   b. The PI must comment how proposed amendment will affect the ongoing trial. Alteration in the budgetary status, staff requirement should be clearly indicated and the revised budget form should be submitted.
   c. If the amendments require a change in the consent form, the copy of revised Consent Form should be submitted to Ethics Committee for approval. If the amendment demands a re look at the toxicity or side effects to patients, the same should be documented.
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   g. Any deviation/violation/waiver in the protocol must be informed.

STANDS APPROVED UNDER SEAL

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CERTIFICATE - II

This is certify that this dissertation work titled **"ASSESSING TIME SINCE DEATH BY USING CHANGES IN ELECTROLYTES IN C.S.F AND VITREOUS HUMOUR IN BODIES SUBJECTED TO AUTOPSY"** of the candidate **Dr.Rama.V,M.B.BS, D.M.R.T**, with registration Number **201524101** for the award of **M.D.** Degree in the branch of **FORENSIC MEDICINE (XIV).** I personally verified the urkund.com website for the purpose of plagiarism check. I found that the uploaded thesis file contains from introduction to conclusion page and result shows **1 PERCENTAGE** of plagiarism in the dissertation.

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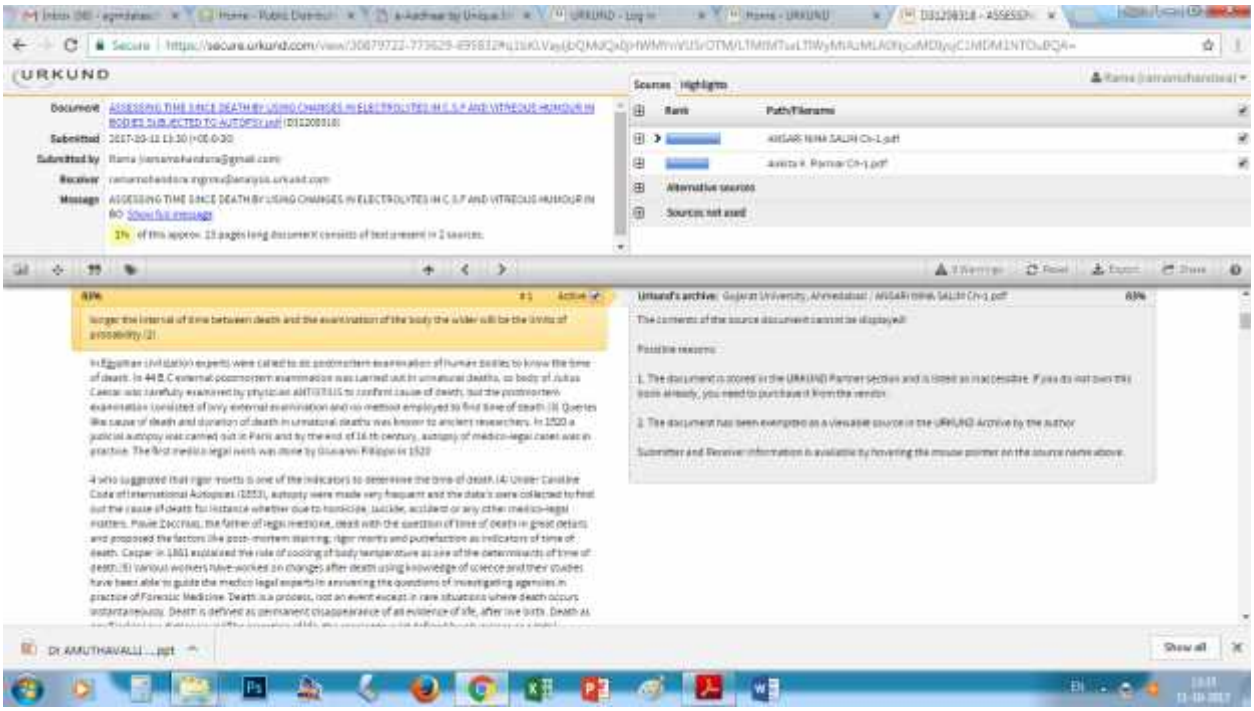
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INTRODUCTION

Life and death are inseparable and it is well-known, that death is definite in every one's life, and it is inevitable. Its occurrence is also unpredictable. For all living things in the world death is definite.

The tolling of a bell announcing the death is known as death knell, death is an omen or destruction of every human being. But a death knell for a person when, why, where and how differs for everyone. It may come in the form of natural or unnatural causes, unnatural in the form of homicide, suicide and accident. To find the cause of death scientifically and technically has become essential in this scientific era. So comes the importance of Forensic Medicine.

A Registered Medical Practitioner will have to give evidence as a medical expert in the court of law to prove the guilt or innocence of an accused. He plays a role of a responsible and reliable source of evidence, on which the liberty or penalization of the accused depends.

While investigating a crime, it is essential for the investigating officer to have adequate knowledge about the place of death, cause of death and "time since death" (TSD). TSD is the time interval between the death and postmortem examination also called as Postmortem Interval (PMI). In criminal cases the PMI serves as an important clue to the Investigating Officer to probe and helps them to eliminate the innocent. The importance of PMI is to aid in identification

of an accused, and in cases of violent death, limit the number of suspects and helps to validate or reject an alibi and verify witnesses' statement. In civil cases also the time of death plays a vital role in deciding about the legal heir ship, inheritance / succession of the property.

To fix time since death based on Postmortem changes alone, has been a constant challenge to medico-legal workers in many situations. The time of death becomes very important in certain situations. And this question is raised to most of the Forensic experts by the Investigating Police Officers, sometimes with touch of accuracy. Though it is not possible to fix up exact time of death by any autopsy finding, yet some close and reasonable approximation should always be aimed at. Theoretically much reliance may be placed upon both physical and chemical changes that take place after death but in practice these changes are never constant but variable. Determination of accurate time since death is extremely difficult as timings of onset and the rates of postmortem changes are usually governed by unpredictable endogenous and exogenous factors such as age, sex, nutrition, cause of death, place of death, climatic conditions temperature both inside and outside the body etc… apart from individual biological variation of onset and rate of change. Therefore the autopsy performing Medical Officer should wisely avoid making dogmatic opinion regarding postmortem interval and preferably mention it in ranges.

Estimating time since death as per CAMPS(1), was by a reasonable guess, taking into account all known factors, and our aim should be to limit the margin of error inherent in assessing the effect of these factors. At the same time repeated experience teaches the investigators to be wary of in relying on any one single observation for estimating the time since death.

To fix the time since death within the limit of probability is a recurring problem in Forensic Medicine. It is self-evident that longer the interval of time between death and the examination of the body the wider will be the limits of probability.(2)

In Egyptian civilization experts were called to do postmortem examination of human bodies to know the time of death. In 44 B.C external postmortem examination was carried out in unnatural deaths, so body of Julius Caesar was carefully examined by physician ANTISTIUS to confirm cause of death, but the postmortem examination consisted of only external examination and no method employed to find time of death.(3)

Queries like cause of death and duration of death in unnatural deaths was known to ancient researchers. In 1520 a judicial autopsy was carried out in Paris and by the end of 16^{th} century, autopsy of medico-legal cases was in practice. The first medico legal work was done by Giuvanni Fillippo in 1520

who suggested that rigor mortis is one of the indicators to determine the time of death.(4)

Under Caroline Code of International Autopsies (1553), autopsy were made very frequent and the data's were collected to find out the cause of death for instance whether due to homicide, suicide, accident or any other medico-legal matters. Paule Zacchias, the father of legal medicine, dealt with the question of time of death in great details and proposed the factors like post-mortem staining, rigor mortis and putrefaction as indicators of time of death. Casper in 1861 explained the role of cooling of body temperature as one of the determinants of time of death.(5)

Various workers have worked on changes after death using knowledge of science and their studies have been able to guide the medico legal experts in answering the questions of investigating agencies in practice of Forensic Medicine.

Death is a process, not an event except in rare situations where death occurs instantaneously. Death is defined as permanent disappearance of all evidence of life, after live birth. Death as per Black's Law dictionary is "The cessation of life; the ceasing to exist defined by physicians as a total stoppage of circulation of the blood and a cessation of animal and vital functions consequent there upon, such as respiration, pulsation etc. After death there is

complete cessation of respiration and circulation, with the results certain changes appear in the tissues and cells of the body. These changes can be reflected in the tissues grossly, microscopically and chemically. In some cases cause of death plays an important role, whereas in others time since death plays a vital clue for investigation. So to fix exact time of death is a great challenge to Forensic experts. Time since death has also become important in case of planning for resuscitation and organ harvesting.

Different methods to estimate post-mortem interval are cooling of body, post-mortem lividity (physical process), rigor mortis (physio-chemical process), decomposition changes, and circumstantial evidence. Based upon the above observations only, approximate time of death can be assessed. But the judiciary is in need of fixing exact time of death by some other methods, so we have to use some other methods to improve the accuracy of TSD.

Many biochemical changes begin to take place in the body immediately or shortly after death and progress in a fairly orderly way until the body disintegrates. Each change has its own time factor or rate. These changes occur in various body fluids including vitreous humor of eye and CSF. Therefore determining the chemical changes could help us to ascertain time since death more precisely.

These biochemical changes by far and by large are being used to determine the postmortem interval in recent years. Various body fluids which are available for the chemical examination are whole blood, serum. CSF, aqueous humor and vitreous humor. Among these the most widely used method is estimation of electrolytes in vitreous humor and CSF, and among the electrolytes potassium concentration gives a more precise value of time since death.

AIMS AND OBJECTIVES

1. To know the relationship between changes in electrolytes [potassium, sodium and chloride] in vitreous humour and cerebrospinal fluid with increasing postmortem interval.

2. To find out the correlation between time since death and changes in electrolytes in vitreous humour and C.S.F.

3. To study if there is any correlation between cause of death and its changes with electrolytes.

4. To find if there is any relation between age of the person at the time of death with changes in concentration of electrolytes in vitreous humour and C.S.F.

REVIEW OF LITERATURE

Nauman et al (1959) studied on 211 post-mortem cases. He found that there is rise in vitreous potassium values after death, but did not corelate it with post-mortem interval. He also observed that there is an average rise of potassium concentration in vitreous of 7.2 mg/dl with a postmortem interval of 9 hrs.

Jaffe (1962) analyzed vitreous humour of 31 cases, none of them with previously diagnosed electrolyte imbalance or any other medical condition related to elevated potassium concentration. He found a consistent rise in the level of potassium starting shortly after death and continuing for 125 hours. He also observed that there is no significant difference between refrigerated bodies and those kept at room temperature.

Adelson, Sunshine, and Rushforth et al (1963) used 349 samples from 269 cases. In 40 bodies the vitreous humour was obtained from both the eyes at the same time. No significant difference was noted in the potassium levels of both the eyes, determined by flame photometry. They found a straight line relationship between vitreous potassium concentration and postmortem interval. They also noted that there was a wide variation in the potassium values of samples obtained from individuals dying of chronic illness in comparison to those succumbing from acute trauma. They established that there were

individual variations in the slope of potassium rise which were independent of environmental factors. The slope of the group as a whole was 0.17 mEq/l and the 95% confidence limit for all cases was $\pm$ 10 hours.

Sturner (1963) alone and later Sturner and Gantner (1964) in a more detailed study, reported on 54 coroner's and 37 hospital cases. In 15 of these cases, vitreous humour was drawn from both the eyes simultaneously and the average difference between the two eyes in potassium concentration was 0.1 mEq/l in the 54 coroner's cases, there was a linear relationship of the potassium values obtained by flame photometry and the postmortem interval. There was a slope of approximately 0.14 mEq/l and the standard error was $\pm$ 4.7 hours which did not appear to increase with an increasing postmortem interval. Environmental differences of temperature were not felt to be important within a narrow range. On the other hand a greater variability was noted in specimens obtained from hospital cases than in the more acute deaths encountered in coroner's cases.

From these data Sturner developed a formula for determining the postmortem interval in hours:

PMI(hrs)= 7.14 x k+ (mEq/L) - 39.1

Where: - PMI =Postmortem interval

K+ =Vitreous humour potassium concentration

This equation has been widely quoted and extensively used by forensic pathologists.

Hughes (1965) studied 135 cases out of which 55 were of sudden deaths. She found identical values between vitreous humor drawn from both the eyes at the same time, but did not give the number of cases studied. Her studies showed 95% confidence limits of approximately $\pm$ 20 hours, which were of greater variation than that of Stunner (1964).The values of her graph of sudden death cases suggested a biphasic linear pattern.

Hansson, Votila, lindfors et al (1966) analyzed vitreous potassium value of 108 cases with postmortem interval not exceeding 250 hours. The method used for analyses was flame photometry. They found that the vitreous potassium increased up to 120 hours and then leveled off. The slope during the period when potassium levels were increasing was 0.17 mEq/l per hour with an intercept of 8mEq/l. The 95% confidence limit was over $\pm$ 20 hours.

Leahy and Farber (1967) worked on 52 cases and found values of vitreous potassium ranging from 4.44 to 16.6 mEq/l. No mathematical relationship was found between vitreous potassium concentration and postmortem interval in the 12 cases dying of sudden death.

Lie (1967) studied 88 hospital cases using the formula proposed by Sturner giving a slope of 0.14 mEq/l per hr. The method used to determine vitreous potassium was flame photometry. There was a linear rise in potassium levels with increasing postmortem interval. He found a good relationship between the actual and expected values in majority of his cases. Differences more than 1.0 mEq/l occurred in only four case. Out of this 88 cases, in 20 cases the samples from both eyes taken at same time showed no appreciable difference. He stressed the necessity of withdrawing all fluid possible from the globe and avoiding forceful expiration of vitreous as an important factor in getting accurate results.

Coe (1969) studied 160 cases, all having normal electrolyte value prior to death. He found a linear rise of vitreous potassium level with increasing postmortem level. With an increasing Postmortem interval up to 100 hours, but found this to be biphasic. There was more rapid rise in first few hours after death. In the first 6 hrs, the slope was 0.32 mmol/l per hour with an intercept of 4.00 mmol/L, for the remainder of cases with postmortem interval over 6hr the slope was 0.1625 mmol/l per hr, with an intercept 6.19 mmol/l. In more than 100 individual's potassium values were obtained at different postmortem intervals for each eye, and this showed that the rate of rise had marked individual variations with the slope varying from 0.085 to 0.450 mEq/l of

potassium per hour. Thus agreed that the standard error increased with increasing postmortem interval as per Hansson. In 20 cases vitreous humour was taken from each eye at same time and analyzed independently. The values were nearly identical in the two eyes even though several days elapsed between deaths and collecting the sample.

Adjutantis and Coutselinis (1972) established a linear relationship between vitreous potassium and postmortem interval, but the slope steeped at 0.55mmol/l per hour for the first 24 hrs and then leveled off. These authors took specimens from two eyes at different times. The postmortem interval was then determined by having the slope developed from these two values pass through the normal vitreous potassium value of 3.4mmol/l at zero hours. By this method the author hoped to overcome individual variation in the procedure and explained an accuracy of $\pm$ 1.1 hrs, in first 12 hrs after death.

Crowell and Duncan (1974) studied on 60 cases and related their vitreous potassium concentration to the log of postmortem interval after Jaffe, who did it in 1962.They presented a data with 95% confidence limit of approximately 12 hrs.

Komura and Oshiro (1977) performed experiment on 90 cadavers to study the influence of potassium in vitreous with increase in postmortem

interval. They found a linear relationship between vitreous potassium concentration and postmortem interval.

Gregora, Kratochvil et al (1978) observed the proportion of potassium concentration in vitreous humour in 47 deceased bodies. They found increase of potassium in linear proportion to the time elapsed from death. The simultaneous estimation of the proportion of potassium in vitreous humour enabled more precise ascertainment of time of death.

Blumenfeld, Mantell and Catherman et al (1979) studied in 127 children their vitreous humour and found that the potassium in vitreous increased with increasing postmortem interval in a linear fashion with 95% confidential limit up to 26 hrs. They concluded that the vitreous humor potassium concentration cannot be used to establish the time of death.

Foerch, Forman and Vye (1979) in a report found a linear relationship between vitreous potassium concentration and postmortem interval but the slope of line and the 95% confidence limit were not provided.

Forman and Butts (1980) also found a linear co-relation between potassium and postmortem interval but neither gave the report of slope of potassium increase nor 95% confidence limit in their study.

Henry and Smith (1982) reviewed the post mortem interval by chemical analysis on blood and Cerebro spinal Fluid. They inferred an importance in early postmortem interval. The single most accurate method to determine PMI is the potassium content of vitreous humour, which shows a linear rise with post mortem interval of 12 to 100 hours after death. They explained that the rate of vitreous potassium rise is fairly independent of environmental influence.

Mason, Harkness, Elton et al (1983) studied the dead and found the postmortem elevation of vitreous humour potassium concentration but were unable to get sufficient linearity with increasing postmortem interval with any degree of accuracy .

Schoning and Strafuss (1984) summarized the effect of time and temperature on postmortem vitreous humour potassium concentration from 60 adult's cadaver. Ante mortem and postmortem vitreous was analyzed for electrolytes (potassium) level, which rose with increase in time after death. And Sodium levels remained below ante mortem level.

McKoy and Choo han (1985) analyzed 105 cases and found a linear relationship between vitreous potassium concentration and the postmortem level. However, this was found to be biphasic, having a steeper slope in early hours of postmortem than the later hours.

Balasooriya, Hill and Williams (1986) analyzed 70 vitreous humour from both eyes collected at the same time after the death. They found that there were significant changes ($p < 0.00001$) in vitreous humour potassium level after death. They concluded that there is a gradual linear increase in potassium concentration in vitreous humor from the time of death. Potassium determination was made on a sequential multichannel analyzer using potassium ion selective electrode.

Bray (1986) studied the postmortem chemistry of vitreous humour to assess the time of death. The rate of rise of potassium was diminished in chilled eye. They also observed that Freezing and re-warming caused a sudden rise in potassium levels.

Coe and Apple (1987) studied in 48 autopsies, the difference in vitreous potassium values with different instrumentation and demonstrated that potassium concentration obtained by FLAME Photometry were lower than the values obtained by direct potentiometry with a potassium ion selective electrode.

Farmer and Benomran et al (1988) determined the levels of potassium and sodium in postmortem vitreous humour from 61 human controls, 13 fire fatalities. The effects of internal changes, time related external parameter

and different causes of death evaluated. Despite the positive correlation and marked increase of potassium and to a lesser extent of magnesium with the length of increase in postmortem time, individual biological variation limited the usefulness of prediction of postmortem interval based on electrolyte data .Vitreous potassium concentration were affected by external influences of fires which increase the rate of release of potassium.

Stephen and Richards (1989), they disagreed the usefulness of vitreous humour Potassium concentration as a predictor of the post mortem interval. They studied over 1427 cadaver of all with quantified ages.He studied the vitreous sample with direct potentiometry with a potassium ion selective electrode. The vitreous potassium showed a linear increase with an increasing postmortem interval. The slope was 0.238 mEq/l/ hr with a zero intercept at 6.342 mEq/l. The 95 % inverse prediction interval was approximately 20 hrs .The linear regression equation for the data was y= 0.238 chi + 6.342, with a coefficient of determination (r2) of 0.372 .This (r2)of value mean that 62.6 % Of the variation of potassium is unaccounted for by the variation in postmortem interval .They stated that further studies were required to attribute this unaccounted variation to quantifiable factors .This could narrow the inverse prediction interval and enable vitreous potassium values to be useful aid in the prediction of postmortem interval.

Madea, Henssege and Honnig et al (1990) studied 170 cases including sudden and hospital deaths after chronic disease, whose time since death was accurately known. In 170 cases the vitreous was drawn and electrolytes potassium and sodium determined. They found that 170 cases showed linear relationship between the mean potassium values of eye and time after death up to 120 hours. The relationship between the concentration of potassium and the time of death was mainly influenced by ante mortem electrolyte imbalance caused by disease and duration of terminal episode. The influence of terminal episode was best identified by its duration. In order to have a method suitable for every case and to be precise as possible they looked for parameters in vitreous humor which was stable in post mortem and indicate ante mortem electrolyte imbalance. Their investigation on potassium in vitreous humor, including sudden death and chronic lingering disease revealed 95 % limits of confidence from 34 hours up to 120 hours of postmortem interval. Reviewing only cases (138) with no previous electrolyte imbalance, the 95% confidence limits is reduced to 22 hours.

Madea and Henssage (1995) studied cases of sudden death from trauma and natural causes of death for estimating time interval since death by measuring potassium in the vitreous humor. In all cases sodium and chloride are stable in the postmortem interval up to 120 hours. Temperature outside is

less than 50degree F. They explained that the presence of metabolic disorder would result in increased level of potassium in the vitreous humour before death, making postmortem time estimation falsely high and unreliable.

Madea et al proposed a formula to estimate postmortem interval in hours as: -

PMI (hrs) = 5.26 x k (mEq / L) – 30.9

Where PMI is – postmortem interval

K+ - Vitreous humor potassium concentration

This formula has the 95% confidence limit of + 19 hours over estimate the time since. They also found that Sturner formula tends to overestimate the time since death due to lesser slope.

James, Hoadley and Sampson (1997) found that the estimation of postmortem interval by analyses of vitreous humour had certain advantages over analyses of blood and cerebrospinal fluid. They explained that potassium had shown to increase in concentration in vitreous in a linear fashion after death .In their study they measured vitreous potassium in 100 subjects with known postmortem intervals. They used Simple linear regression analyses on the data collected .It was observed that using potassium as a measure to estimate the post mortem interval were associated with increased accuracy.

Ferslew, Hagardon and Harrison et al (1998) used Capillary ion analysis (CIA) technique for direct detection of potassium concentration in human vitreous humor. CIA is a capillary electrophoresis method which uses the differential electrophoretic mobility of ions to perform a separation of an ionic mixture. Coefficients varied from 1.45 to 13.8 %. Application of this methodology to 25 vitreous humour from autopsy cases was compared to analysis by ion specific electrode for potassium concentration comparison coefficient of 0.9642.CIA is applicable to forensic analysis of potassium concentration in forensic vitreous humour. Quantification of numerous cation concentration is possible by direct CIA of vitreous.

Pounder, Carson and Johnston et al (1998) analyzed between both eyes the electrolyte concentration in 200 medico-legal autopsies cases using vitreous humour. He divided the cases into 3 groups as per their potassium concentration that is first group < 15 mmol/l , second group 15 – 20 mmol/l , and cases with > 20 mmol/l as third group. Mean vitreous Sodium concentration for all cases (n=200) was 112 to 173 mmol/l. (mean 148, standard deviation = $\pm$ 8.9) between the eyes the difference were 0.8 mmol/l. Mean paired vitreous chloride for all cases was 73 to 123 mmol/ , mean 109 ;SD =$\pm$7.8, between eye concentration difference of sodium and chloride were tolerable .The significant

and erratic differences in vitreous potassium undermined the usefulness in estimation of time since death .

Tagliaro, Manetto and Cittadine et al (1999) studied the analysis of potassium in vitreous humour which is long been regarded as an important tool in medico legal and forensic toxicological investigation, particularly for determination of postmortem interval. Their work was aimed at the optimization and validation of a reliable, simple and fast capillary electrophoresis method for potassium analysis in the vitreous humour with indirect UV detection at a wavelength of 214nm.The method followed was electrophoretic separation with constant voltage run. This method showed good linearity in the concentration range from 6.5 mmol to 16.26 micromol, with an r2 value (coefficient of determination).

Munoz, Suarez –Penaranda et al (2002) recorded that there were many formulae available to estimate the relation between the potassium concentration in the vitreous humor and the post mortem interval. Typically, it was based on a correlation test and linear regression using the postmortem interval as the independent variable and potassium concentration as dependent variable in order to estimate the confidence interval. However, their studies proved that more precise measurement of PMI can be obtained if potassium concentration is considered as an independent variable. The lines of regression obtained from

the most recent deceased subjects with forensics received for autopsy formed a formula

$$K+ = 5.589 + O.174 \text{ PMI.}$$

In this study they proposed an extra factor that is the cause of death which also modifies the relationship in estimating time since death. In case of hanging deaths the results to some extent matched with time since death compared to other causes of death. The slope was less and the precision was obviously enhanced.

Prasad et al [15] in 2003 studied correlation of K+ level of vitreous and the postmortem interval and found that the rise in K+ level after death has a strong correlation with the Post-mortem intervals.

Singh et al [14] in 2005 studied 1026 subjects in which 698 were males and 328 were Females. He studied only on those subjects in whom time, mode, manner, cause of death and other demographic profiles were precisely known and the dead bodies kept at room temperature were only taken into consideration. Subjects with significant ante-mortem electrolyte imbalance or on diuretics were excluded from the study. Measurement of Na+ and K+ was carried out after centrifugation using flame photometry. They found that the mean vitreous Na+/ K+ ratio was slightly more in the left eye (13.50 ± 5.27)

then in the right eye (13.48 ± 4.95), however this difference was found to be statistically insignificant ($p < 0.05$).

Mulla A [16] study in 2005 hypothesized that the concentration of vitreous biochemical constituents in the same pair of eyes change at the same rate and this change that occurs in a time dependent fashion may be utilized in accurately estimating the POST-MORTEM INTERVAL.

Thierauf et al (2009) did a study and his aim of study was a methodical investigation of two methods of sample. The method used were Centrifugation and Ultrasonic bath. The parameters studied were Na +, K+ and Cl-. Analyses were performed by photometrically or ion – selective electrode. Regarding the two pretreatments methods, there was no significant difference in the concentration. The concluded that there were no significant differences in potassium concentration in both the eyes.

Modi et al in his 22 edition (2011) has mentioned that the potassium level in vitreous humour progressively increases after death. Any way he has not mentioned any formulae.

Rao et al (2003) has explained changes in biological fluids after death, potassium level in vitreous humor is found to increase up to 100 hours after death. And chloride in C.S.F decrease, a concentration below 440 mg indicates PMI is less than 25 hours.

S.K Singhal et al (2004) has mentioned in his biochemical changes in bodily fluids that after death level of potassium increases in vitreous humour in correlation with postmortem interval.

H.M.Cox et al has applied biochemical methods in estimating time since death rather than using rigor-mortis and hypostasis as device to estimate time since death. The use of blood for such analysis was considered useless as the original state of blood is rapidly altered after death. Therefore, the fluids employed in an attempt to device chemical methods are usually separated from the vascular system such as the cerebro spinal fluid and the intra ocular fluid. He inferred that in vitreous humour the potassium increased in concentration and sodium relatively decreased. In CSF similarly there was increase in potassium and sodium remains unchanged.

B.V.Subramanium et al observed that postmortem increase of potassium in CSF upto 15 hours after death. He also claimed that potassium level increases between 12 to 72 hours after death (0.152mEq/c/hr}.

Rajnikanta and S.R.Singh et al (2012) did a study on postmortem biochemistry in vitreous fluid and C.S.F. They intended to compare the accuracy of estimating the postmortem interval with biochemical parameters of vitreous fluid and C.S.F. The fluid was collected from 100 autopsy cases with

known time since death. Their study concluded that vitreous fluid was better fluid than CSF comparatively for estimating post mortem interval. It was also observed that among the fluid, level of sodium and potassium in vitreous humour are giving more accurate results in comparison to CSF Finally, potassium concentration in vitreous humour is a single best parameter to estimate post- mortem interval.

Nidhi Sachdeva, Yashoda Rani et al (2011) used biochemical estimation to assist precise estimation of time since death. Sophisticated, automatic, biochemical methods were used to determine time since death. Also he chooses vitreous humour out the various body fluids to study post-mortem interval. Among the electrolytes k+ levels in the post-mortem period has been considered to be helpful in determining the postmortem interval. The time dependent rise of vitreous k+, has been considered to be helpful in post mortem interval estimation. However, he concluded that since there are many lacunae in present study, further research is needed to make a conclusion.

Chaitanya, S.Kulkarni and Gajanan et al (2013) estimated post mortem interval using electrolyte especially potassium in vitreous humour. They studied in 200 cases posted for medico legal autopsy whose time of death was known. They concluded that there was a linear relationship between vitreous humour potassium and postmortem interval. They also observed that rate of rise

of vitreous potassium level in vitreous was 23 mEq/l/hr . No significant difference in vitreous potassium concentration between the two eyes. Also there is no significant effect of age, sex, temperature and mode of death on vitreous potassium level after death.

Jayanthi Yadav, Aashish Deshpande et al (2007) in 100 medicolegal cases subjected to autopsies with known time since death, estimated time since death using sodium and potassium ion concentration levels in C.S.F. The study result revealed a significant correlation of sodium and potassium ions in CSF up to 25 hours. Since death, with average rise per hour 1.21 meq/h for sodium ions. They also observed a useful relationship between sodium, potassium ion ratio and PMI. The study concluded that changes in CSF electrolyte is a significant parameter to estimate time since death.

Nidin Barmate et al (2014) sampled about 201 cases subjected to autopsy, to study the electrolytes in vitreous humor with known time since death to estimate the time since death. The objectives of study was to estimate the postmortem interval with level of vitreous potassium and sodium. And correlation of postmortem changes with changes in vitreous humour. They concluded that there is a linear relationship between vitreous humor potassium concentration and postmortem interval. The estimation of Postmortem interval using vitreous potassium is:

$$PMI = 11.63(K) – 70.90$$

The average rate of increase of vitreous potassium was calculated as 0.175mmol/L. They also concluded that accuracy of PMI calculated using death changes is less significant and less accurate.

Baniak N, Mulla et al (2015) studied application of vitreous potassium concentration in postmortem cases to assess the time since death. They concluded that great progress has been made in utility for determining PMI. Potassium is the most extensively used electrolyte to determine PMI. They also inferred that between the eyes the difference in vitreous humour is not statistically significant.

S.K.Gupta et al (2015) studied the relation between the potassium concentration and PMI. Formulae's based on correlation test and linear regression test are available to calculate PMI using PMI as independent variable and K+ as dependent variable. This formula helps us to estimate confidence limit. In Forensic aspect it is necessary to use K+ as the independent variable to estimate the PMI. They concluded that PMI obtained by these formulae are an inexact approach, and leads to false estimations. So they wanted to change the variables, and obtain a new equation in which K+ is considered as the independent variable and PMI as dependent. The regression line obtained from

our data is [k+] = 5.35 + 0.22 PMI, by changing the variables we get **PMI = 2.58[k+] – 9.30.** They also reported that in non- hospital deaths the results were more favorable. In this case, we get [k+] =5.60 +0.17 PMI, and consequently PMI= 3.92[K+] – 19.04.

FLOW CHART

After Death

↓

Stoppage / cessation of metabolism and energy production

↓

Due to Autolysis

↓

Dissolution of physical and chemical integrity of vitreous body

↓

Cessation of Active Membrane Transparent

↓

Loss of Selective Membrane Permeability

↓

Leads to Diffusion of Ion from Intracellular fluid to

↓

Extracellular fluid

↓

Concentration gradient till equilibrium is reached

Changes in Vitreous humour According to Age

While aging, there is Rheological, Biochemical and Structural alteration in vitreous humour. Rheology refers to the gel-liquid state of the vitreous.

At Birth - Cloquets canals runs straight from lens to optic disc, and contain primary vitreous.

After 4 years – Liquid vitreous appear.

Young age - Vitreous gel appears homogenous and fibrous, and becomes coarse.

45- 50 age- significant decrease in gel volume and increase in the liquid volume, liquefaction begins in the central vitreous.

70- 90 age- More than half of corpus vitreous is liquid.

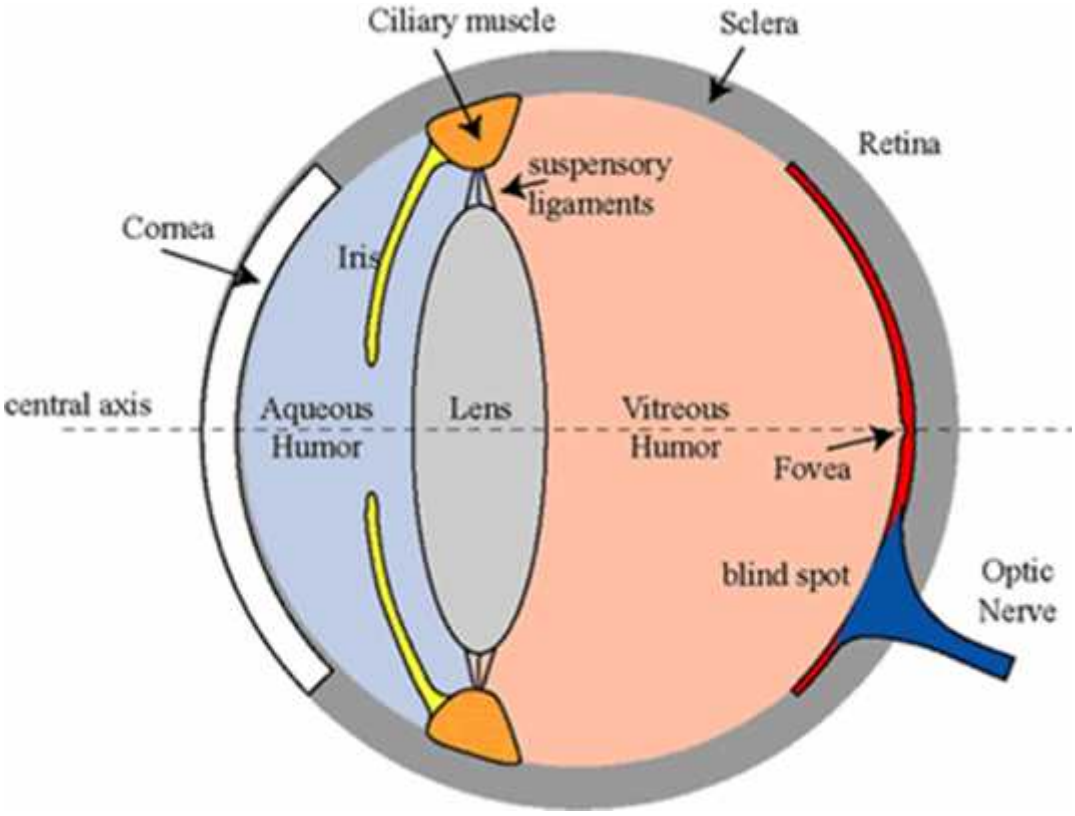
Old age – Secondary vitreous liquefies, shrinks and produce vitreous detachment.

EMBRYOLOGY OF VITREOUS HUMOUR

Development of vitreous occurs in three phases (50)

1. Primary vitreous: seen at optic cup lens vesicle stage. It develops between the third and ninth week of gestation. It is partially surface - ectodermal and partially mesenchymal in origin.
2. Secondary vitreous: formed by the neuroectoderm of optic cup. It develops around ninth week. It consists of collagen and hyalocytes. Primary and secondary vitreous remain in contact with posterior lens capsule as hyaloido capsular ligament.
3. Tertiary vitreous: This forms suspensory ligaments of the lens. This is secreted from the developing ciliary epithelial cells.

ANATOMY OF VITREOUS HUMOUR



The mature Vitreous body (V.B) is a transparent gel which occupies the vitreous cavity in the eyeball. It is almost spherical in shape, except in the anterior which is concave corresponding to the presence of crystalline lens. The outermost part of vitreous (the hyaloid), called the cortex, is divided into anterior cortex and posterior cortex. The vitreous base is a 3 dimensional zone. It extends from 2 mm anterior to Ora serrata to 3 mm posterior to Ora serrata. . The collagen fibrils are especially packed in this region. (51)

V.B is bound anteriorly by the lens and ciliary body and posteriorly by the retina. It occupies four –fifth of the globe. Vitreous humour is an inert colorless, jelly like, hydrophilic gel that serves as an important supporting structure for the eyeball and also functions as optical function. It weighs 4 grams and occupies a volume of almost 4 cc which is approximately two thirds the volume of the globe. Vitreous contains 99% water; rest is solids. The vitreous acts as a gel that surrounds and stabilizes a large amount of water compared with the amount of solids. In human eye, the major part of solid is the glycosaminoglycan that is hyaluronic acid, with molecular weight 3 to 4.5 $x10^6$.

Dissolved in the water of the vitreous gel are inorganic and organic substances. It appears that gradients move in both directions between vitreous and plasma. These gradients are result of several mechanisms, blood ocular barriers, metabolism in the retina and ciliary body, and diffusion process in the vitreous body.

PHYSIOLOGY OF VITREOUS HUMOUR

The normal physiology of vitreous body can be divided into four main groups:

1. Support function for the retina and filling up function of the vitreous body cavity.
2. Diffusion barrier between anterior and posterior segment of eye.
3. Metabolic buffer function
4. Establishment of an unhindered path of light. [49]

TRANSPORT PROCESS IN VH:

The ciliary body pigment holds the Active pump mechanism and the retinal vessels are concerned with active transport of material across the vitreous.

VITREOUS – BLOOD BARRIER:

1. Tight junctional complexes: Between pigment epithelium, retinal vascular endothelium and Non pigmented epithelium of ciliary body. It stops entry of high molecular weight constituents. This is of the greatest importance of all barriers .This barrier restricts flow of serum protein inside ,if this is broken all protein flows in and there is loss of transparency of vitreous humour.

2. Vitreo-Retinal junction at the level of Basal lamina – its function is to inhibit the passage of large molecules.
3. Vitreous cortex: the physio chemical characteristerics of the vitreous hyaluronic acid network.

BIO-CHEMICAL COMPOSITION OF V.H

It is composed of 3 major structural components:

1. WATER (wet weight)- 99%
2. Collagen fibers,
3. Hyaluronic acid (Glycosaminoglycan's) and other minor components.

Solids components composed of 1 % in wet weight, they consist of macromolecular and the low molecular weight constituents.

Macromolecular constituents consist of: Collagen, Hyaluronic acid, soluble proteins.

Low molecular constituents: Sugars, Amino acid and Ascorbic acid.

Concentration of Electrolytes in VH:

Sodium ion (Na): Its concentration in Anterior chamber is almost same as that of plasma and Aqueous humour, that means there is passive diffusion.

Potassium ion (K+: The concentration of potassium in anterior and posterior chamber is more than plasma .This is due to the active transport of potassium across the ciliary body in to the posterior chamber and also because of the active transport through the anterior capsule of the lens and passive diffusion through the posterior capsule of the lens in to the viterous.

Calcium ion (Ca+): Concentration is equal to that of plasma and aqueous humour.

Chloride (Cl-): Concentration of vitreous Chloride is more than that of anterior chamber, posterior chamber and plasma. Since there is exchange of chloride ions across both posterior chamber and retina.

CEREBROSPINAL FLUID

CSF is similar to blood plasma and interstitial fluid.It is present in the ventricular system within the CNS and in the subarachnoid space surrounding the CNS. It bathes both the external and internal surfaces of the brain and spinal cord and provides a protective cushion between the CNS and the surrounding bones.

FORMATION AND CIRCULATION OF C.S.F:

The CSF is produced mainly by the choroid plexus of the lateral, third, and the fourth ventricles, with those in the lateral ventricles being the largest and most important. It is now established that the average rate of CSF formation is 21 to 22 ml/hour or approximately 500ml/day. The mean CSF volume is 150 ml, with 25 ml in the ventricles and 125 ml in subarachnoid spaces. The CSF as a whole is renewed 4-5 times a day.

From the lateral ventricles it passes through the interventricular foramen of Munro into the third ventricle, and then via cerebral aqueduct into the fourth ventricle. Here the fluid escapes via the median aperture (Foramen of Magendie) and lateral apertures (Foramen of Luschka) in the roof of lateral ventricle into the cerebellomedullary and pontine cisterns, respectively. From

here the fluid flows slowly in the subarachnoid space over the brain and spinal cord.

The choroid plexuses, located in floor of the third and fourth lateral ventricle are the main sites of CSF formation. Brain interstitial fluid, ependymal and capillaries may also play a poorly defined role in CSF secretion. The blood vessels in the sub ependymal regions and the pia also contribute to the CSF production. Some substances enter the CSF as readily from the meninges as from the choroid plexus. Electrolytes equilibrate with the CSF at all points in the ventricular and subarachnoid spaces. The transport of sodium, the main cation of the CSF, is accomplished by the action of a sodium-potassium-ion exchange pump at the apical surface of the choroid plexus cells. Ionized compounds such as hexoses and amino acids enter the CSF slowly unless facilitated by a membrane transport system. The CSF space is a dynamic pressure system's pressure determines the intracranial pressure with physiological values ranging between 10 and 15 mmHg in adults.

Production of CSF is a complex process. Some components of the blood plasma, notably water, enter and leave the CSF by diffusion. Others reach the fluid with the assistance of metabolic activity on the part of the choroid epithelial cells. An important factor is active transport of certain ions (notably Na) through the epithelial cells, followed by passive movement of water to

maintain osmotic equilibrium. Transporter proteins in the choroid epithelial cells allow controlled movement of glucose and amino acids into the CSF.

1. **FUNCTIONS:**

The CSF performs the following functions:

1. Fluid Buffer: It serves as a cushion between the CNS and the surrounding bones.
2. Regulating the volume of cranial contents: It acts as a shock absorber, i.e. it prevents the diminishes the transmission of jarring or shocking forces to the CNS. If the volume of blood or brain increases, CSF drains away. If reduced fluid is retained.
3. Maintenance of proper ionic concentration for the neurons of the CNS: Composition of ECF and CSF is almost similar except that in CSF potassium is little lower, Mg+ and Cl- is little higher.
4. Acts as the lymph system in CNS
5. Control of respiration.
6. Medium for nutrient exchange.

MATERIALS AND METHODS

Study conducted on 100 cases brought for medico legal autopsy to Department of Forensic Medicine & Toxicology, Tirunelveli Medical College from May 2016 to May 2017, and the material for study are vitreous humour and CSF. The details of the case including time of death was collected from hospital records.

Inclusion criteria are:

1. Age between 15 to 70 years.
2. Time of death should be known.
3. Sample collection within 24 hours of Death.
4. Cases having clear vitreous humour and C.S. F was taken.

Exclusion criteria were:

1. Injuries involving eyeball and head.
2. More than 24 hours of time since death.
3. Cases of burns.
4. Cases of extensive soft tissues injuries.
5. Age <15 and >70 years.

In this study the cases were divided into 4 groups as per their:

1. AGE
2. GENDER
3. CAUSE OF DEATH and
4. TIME SINCE DEATH

Vitreous humour was drawn from the eye and CSF from either cisternal or lumbar puncture as soon as the body was brought to the mortuary (only clear samples were taken).Details of the patient such as age, sex, post-mortem number, cause of death, date and time of death, time of aspiration of sample were noted. Electrolyte value of sodium, potassium and chloride in vitreous humour and CSF were recorded in the proforma.

Vitreous humour in the posterior chamber of the eye was aspirated gradually, avoiding tear of loose fragments of tissues by needle aspiration through puncture made 5-6 mm away from the limbus using 10ml sterile syringe and 20 gauze needle, the vitreous was replaced by Liquid paraffin to maintain the contour. The sample was transferred to sterile stoppered tube and immediately taken to Central Laboratory, Department of Biochemistry, Tirunelveli Medical College Hospital. Each sample was centrifuged at 300 rmp for 1 min or longer if the sample remained turbid and supernatant fluid was

divided into 2 parts; one part for determination of electrolytes by method of potentiometry with ion selective electrode and another part for determination of chloride by method of Schales and Schales

PROCEDURE IN DETAIL:

METHOD: Potentiometry with ion selective electrode.

PROCEDURE: The machine will calibrate itself automatically and shows ready. Take the sample in Hitachi cup, open the flap, feed the sample until a beep sound is heard. Wipe the probe clean and close the flap. Wait for few seconds and reading of electrolyte is shown in display which is noted.

METHOD FOR DETERMINATION OF CHLORIDE IN CSF & VITREOUS HUMOUR:

PRINCIPLE: The chloride in biological specimen is titrated in an acid medium with standard mercuric nitrate, using s-diphenylcarbozone as an indicator. Chloride ion reacts with mercuric ion to form the soluble but non-dissociated mercuric chloride. At the end point, when a slight excess of mercuric ion is present, a stable violet-blue complex is formed with the indicator. Run the sample, we get the reading of chloride which is noted.

METHODS OF COLLECTION OF CSF SAMPLE

There are two procedures:

1. LUMBAR PUNCTURE
2. CISTERNAL PUNCTURE

1. LUMBAR PUNCTURE

A special needle of 1mm bore and 8cm long with a withdraw able stylet is introduced into the subarachnoid space below the termination of the spinal cord at the level of lumbar vertebra between the 3^{rd} and 4^{th}, or between the 4^{th} and 5^{th}. It is done with the subject lying on his side,and as the body is in rigor the trunk, knees and chin cannot be flexed so approximated to the maximum.

2. CISTERNAL PUNCTURE:

About 2 ml of cisternal fluid was aspirated as a blind procedure, and it is difficult in obese individual and in the body with full rigor mortis. A needle is introduced between the occipital bone and the atlas vertebrae into the cisterna magna. The CSF collected is transferred to a sterile tube and taken to laboratory immediately and analysed, the values in the monitor is noted

Instruments used for analysis of serum is also used for CSF. Since the chemical composition vary for serum and CSF, the question now arises that the method caliberated for serum be applied for CSF as well. So COE has studied extensively to compare antemortem and postmortem chemistry of fluid to form a range of values. The value of different composition of CSF are given below.

COMPOSITION OF CSF

CONSTITUENT(mg/dl)	CSF
Protein	20 – 40
Glucose	40 – 70
Sodium chloride	720 – 750
Na+	137 – 145
K+	2.7 – 3.9
Mg+	2.0 – 2.5
Cl-	116 – 122
Ca+	2.1 – 3.0

RESULTS AND ANALYSIS

In present study, following findings are noted:

TABLE -1

Gender Distribution of cases

S.No.	Gender	No of cases
1.	Male	70
2.	Female	30
	Total	100

The study consisted of 100 samples out of which 70 from males and 30 from females. All in the age group between 20 to 70 years. The same is shown in a pie chart below.

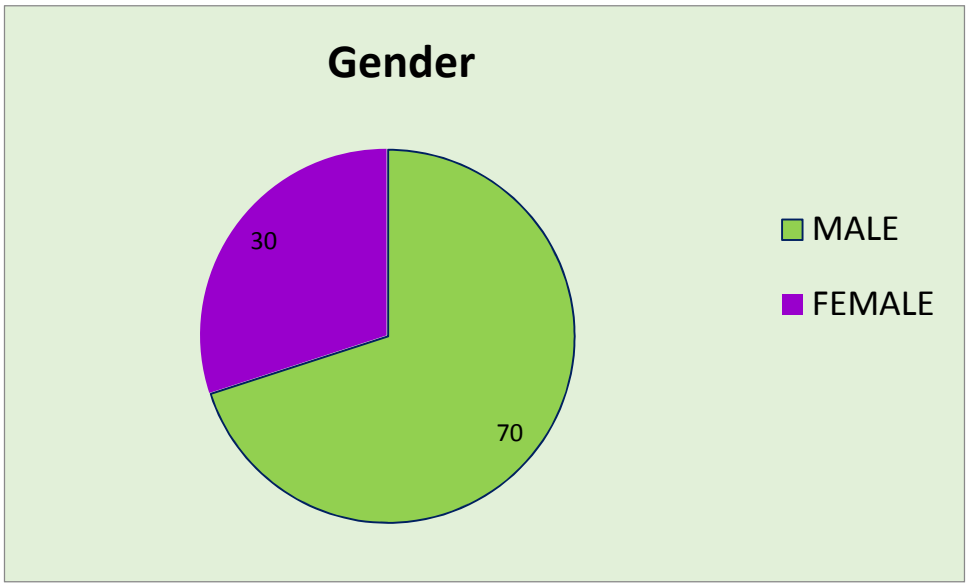


TABLE 2

Showing % Distribution of Cases as per AGE

S.NO	AGE GROUP	MALE	FEMALE	TOTAL	%
1	20 - 29	15	8	23	23
2	30 -39	12	9	21	21
3	40 -49	15	2	17	17
4	50 -59	14	5	19	19
5	60 - 70	14	6	20	20
	TOTAL	70	30	100	100

In my study, the samples were divided into 5 groups as per the age. That is between 20 to 29 years consists of 23% of cases, 21 % of cases fall within 30-39 years,17 % of cases between 40 to 49 years,19 % of cases between 50 – 59 years and 60 - 70 years consisted of 20 % of cases. Each group is further subdivided into male and females.

Age &Sex Distribution Chart

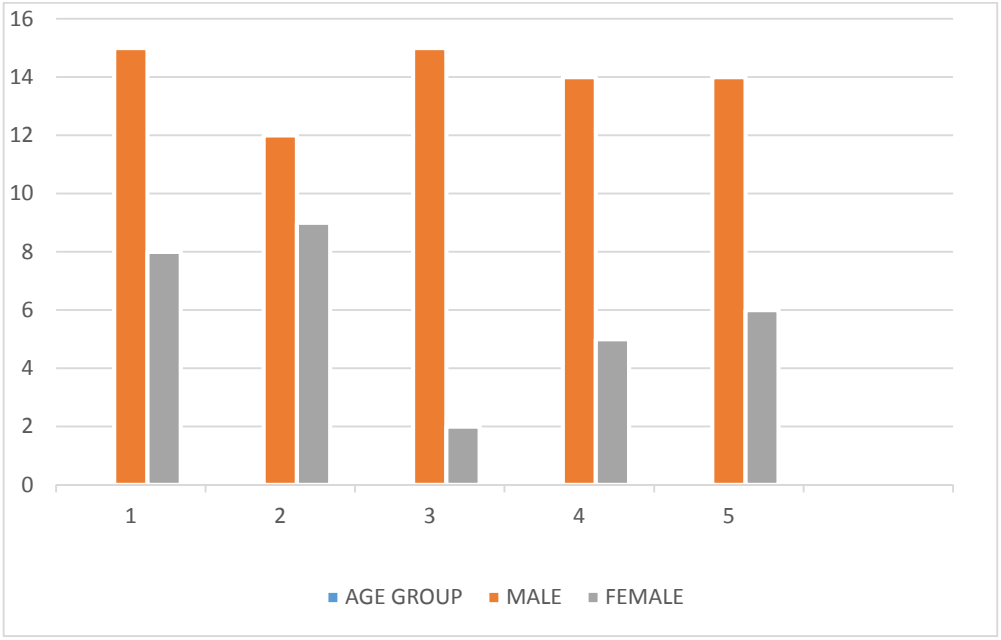


TABLE -3

Showing the percentage distribution of cases as per TSD

S.NO	TSD (hrs)	Total	%
1	0-6	3	3
2	6.1 – 12	29	29
3	12.1- 18	45	45
4	18.1 - 24	22	23
	Total	100	

The sample collection were divided into 4 groups as per the TSD. 3% of my cases belonged to 0-6 H of TSD, 29% of cases belonged to 6.1 to 12 H , 45 % of cases belonged to 12.1 -18 H and 23% of cases belonged to 18.1 -24 H.

The same is shown as bar chart below.

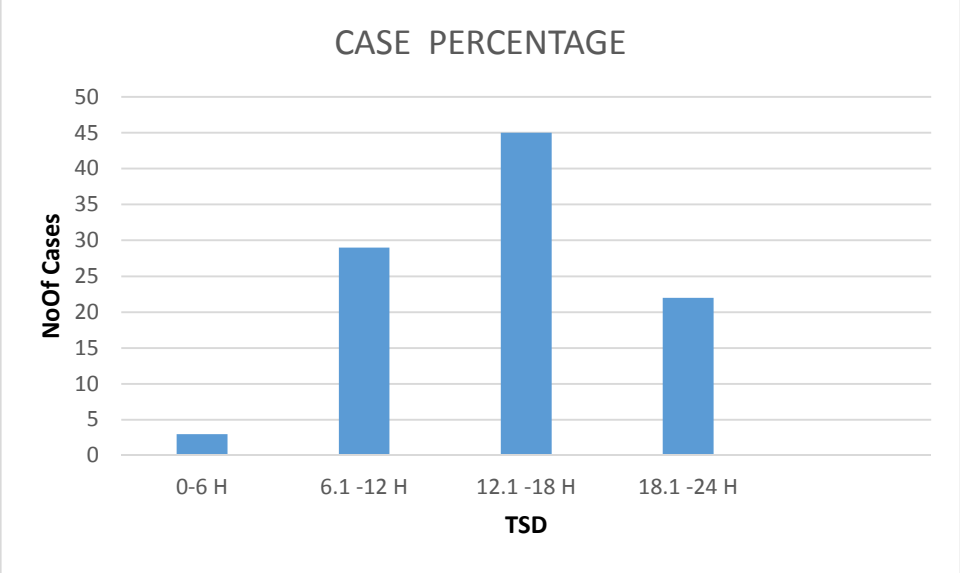


TABLE -4

Showing the distribution of cases in Male and Female as per TSD

S.NO	TSD (hrs)	No. of cases M	F	Total
1	0-6	2	1	3
2	6.1 – 12	26	5	29
3	12.1- 18	27	16	45
4	18.1 - 24	15	7	22
	Total	70	30	100

The total cases were divided into 4 groups as per their TSD and further subdivided into male and female categories. There were 26 male and 5 female cases in TSD 6.1 – 12 H, there were 15 male and 7 female cases in 12.1 -18 H, and there were 15 male and 7 female cases in 18.1 -24 H duration.

The above cases are depicted in bar chart as shown below.

Distribution of cases as per TSD

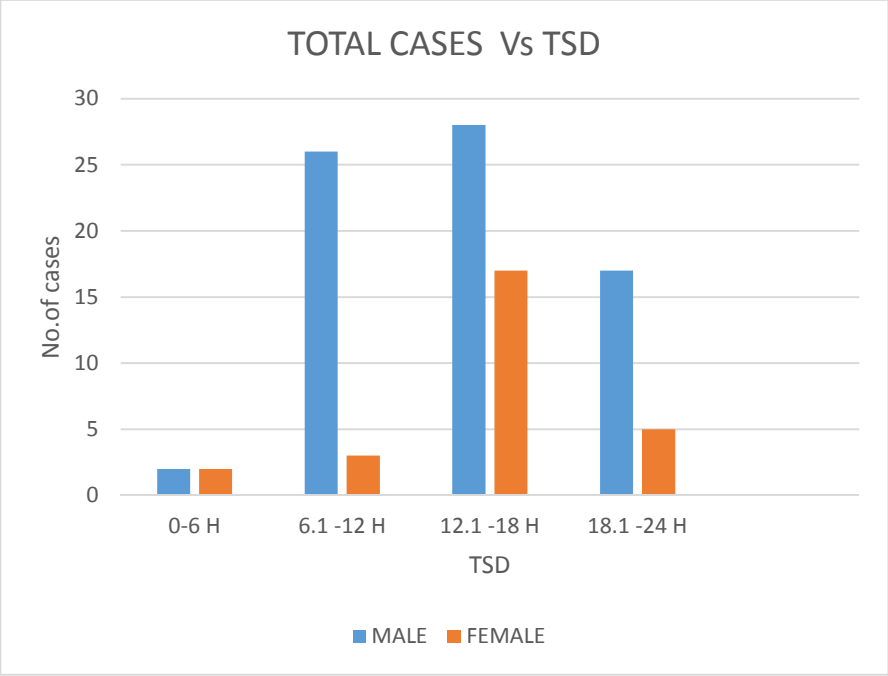


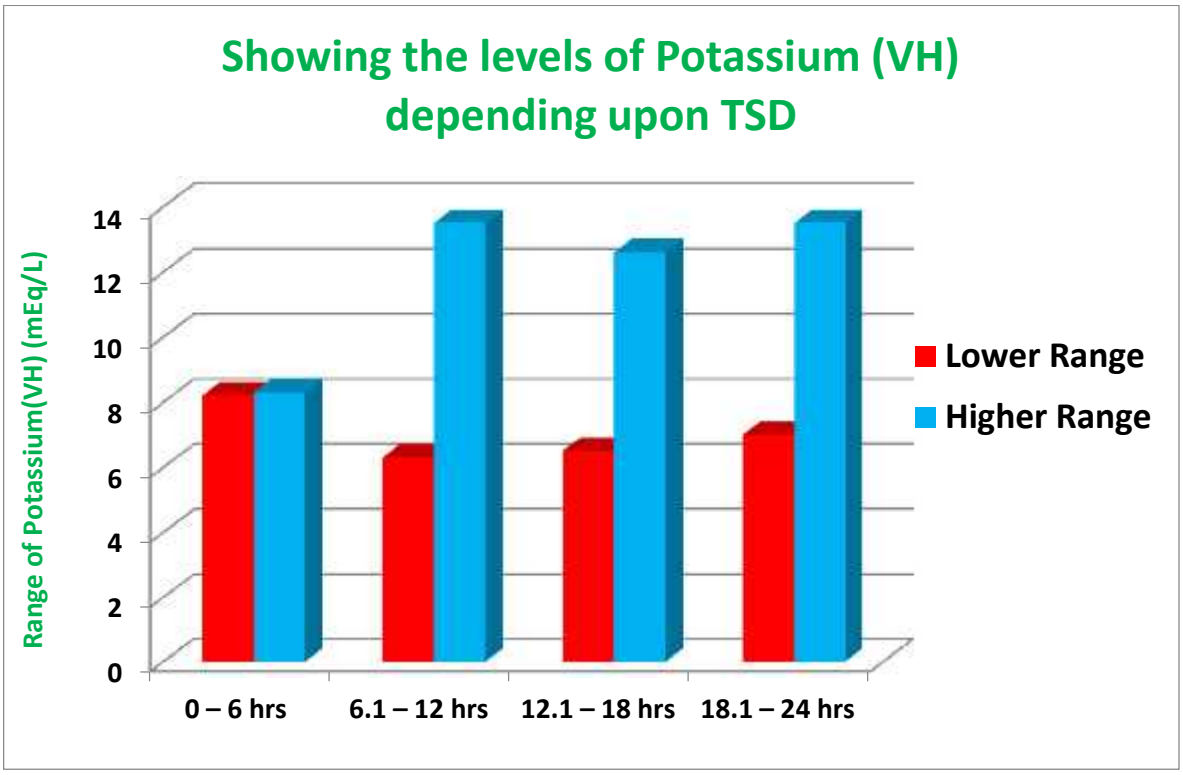
Table No.5

Showing the levels of Potassium (range & mean) in VH depending upon TSD

S.No.	TSD (hrs)	No. of Cases	Range of Potassium (mEq/L)	Mean (±) SD
1.	0 – 6.0	3	8.2 – 8.3	8.23±0.05
2.	6.1 – 12.0	29	6.3 – 13.5	8.87±1.58
3.	12.1 – 18.0	45	6.5 –12.6	9.29±1.51
4.	18.1 – 24.0	23	7.0 – 13.5	10.25±2.03
	TOTAL	100		

The table above shows the distribution of the value of potassium concentration in VH divided into 4 groups as per TSD. The four groups are: 0- 6 H, 6.1 –12 H, 12.1 –18 H and 18.1 – 24 H respectively. In 0- 6 H there were 3 cases with the potassium concentration ranging from 8.2 to 8.3 mEq/L. In 6.1 -12 H there were 29 cases with the potassium concentration ranging from 6.3 – 13.5mEq/L. Between 12.1 -18H there were 45 cases with the potassium concentration ranging from 6.5 -12.6 mEq/L. And in 18.1 – 24 H the potassium concentration is between 7 to 13.5 mEq/L.

The same is depicted in the bar chart below.



Statistical analysis

Comparison	p value for K+
1 and 2	1.000
1 and 3	1.000
1 and 4	0.294
2 and 3	1.000
2 and 4	0.023
3 and 4	0.165

From the above Statistical analysis table it is evident that there is no significant change in the level of potassium concentration in the vitreous humour with increasing time since death.

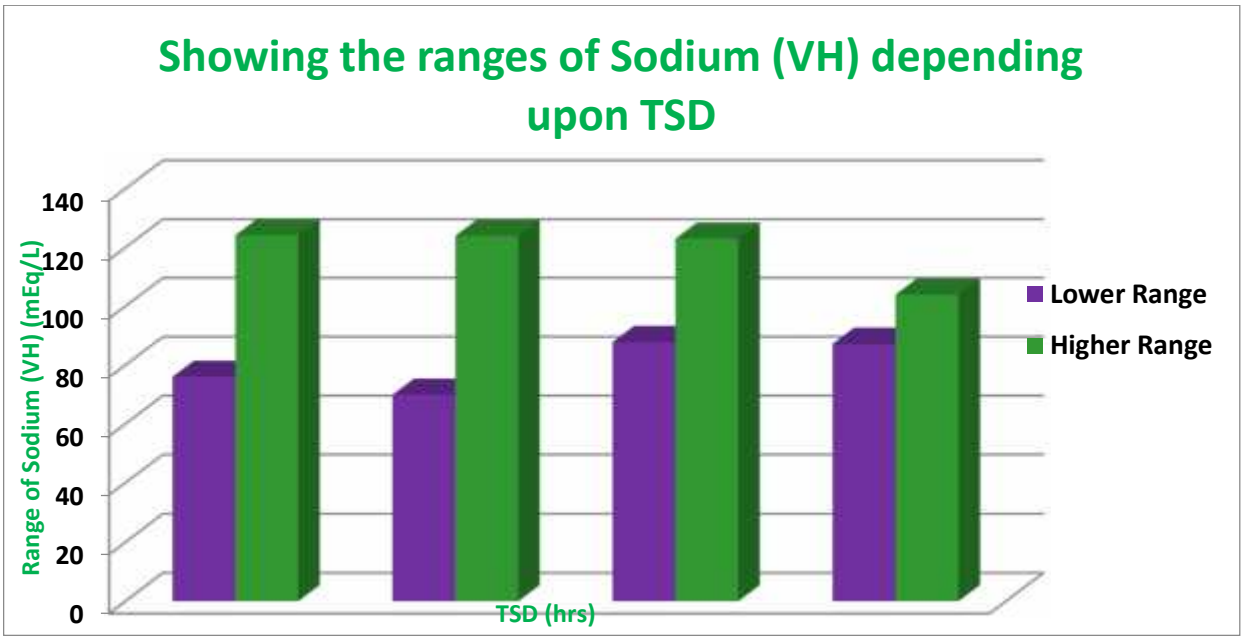
Table No.6

Showing the level of Sodium (mean & range) in VH depending upon TSD

S.No.	TSD (hrs)	No. of Cases	Range of Sodium (mEq/L)	Mean (±) SD
1.	0 – 6.0	3	144 –171	162±15.58
2.	6.1 – 12.0	29	121 – 165	137.62±10.44
3.	12.1 – 18.0	45	120.4 – 158	136.32±5.89
4.	18.1 – 24.0	23	110 – 151	140.89±8.64
	TOTAL	100		

The table above shows the distribution of the values of Sodium concentration in VH divided into 4 groups as per TSD. The value in four groups are: In TSD of 0- 6 H the sodium concentration ranges from 144 -171 mEq/L. In TSD of 6.1 -12 H the range of sodium concentration varies from 121 -165 mEq/L. Between TSD of 12.1 -18H the sodium concentration varies from 120.4 - 158 mEq/L. And in 18.1 – 24 H TSD, the sodium concentration varies between 110 - 151 mEq/L.

The same is depicted in the bar chart below.



Statistical analysis

Comparison	p value for Na
1 and 2	<0.0001
1 and 3	<0.0001
1 and 4	<0.0001
2 and 3	1.0000
2 and 4	1.000
3 and 4	0.224

From this statistical analysis table it is evident that there is no significant change in the concentration of sodium in vitreous humor with increase in time since death.

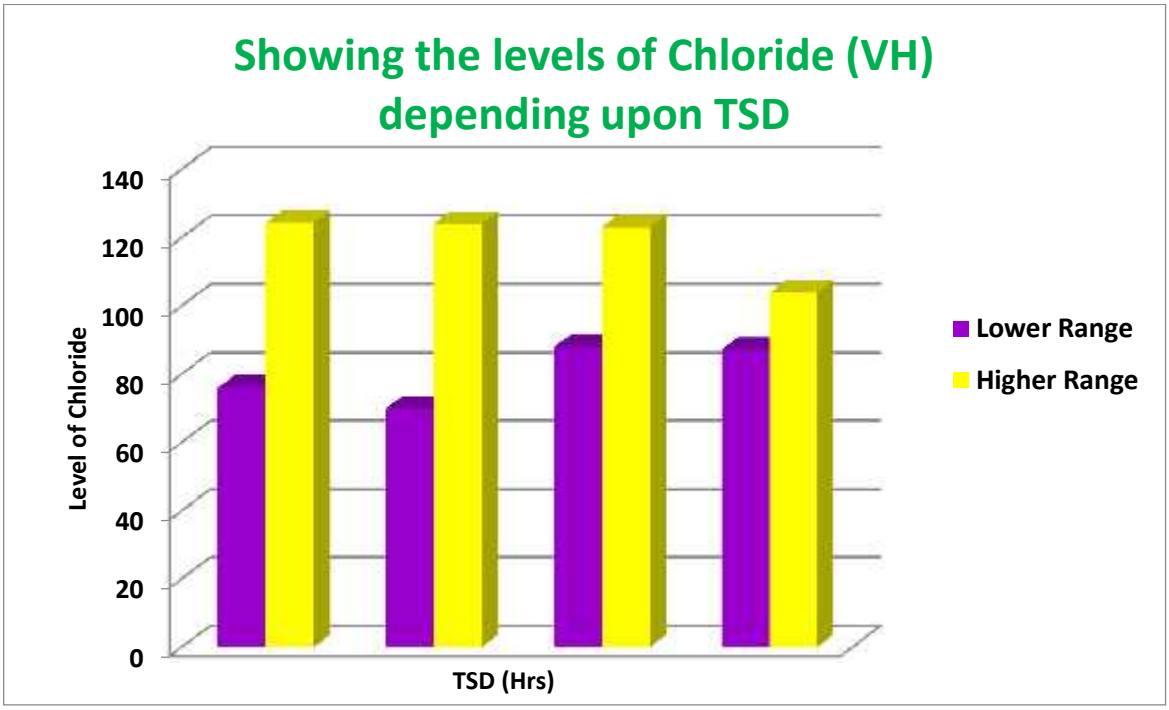
Table No.7

Showing the level of Chloride (mean & rang, in VH depending upon TSD

S.No.	TSD (hrs)	No. of Cases	Range of Chloride (mEq/L)	Mean (±) SD
1.	0 – 6.0	3	106 – 121	116±8.66
2.	6.1 – 12.0	29	61.8 – 121	105.13±15.52
3.	12.1 – 18.0	45	76 – 136.3	103.9±17.87
4.	18.1 – 24.0	23	89.4 – 124	110.64±14.11
	TOTAL	100		

The table above shows the distribution of the values of Chloride concentration in VH divided into 4 groups as per TSD. The value of four groups are: In TSD of 0- 6 H, there were 3 cases with Chloride concentration ranging from 106 - 121 mEq/L .In TSD of 6.1 -12 H, 29 cases with the Chloride concentration ranging from 161.8 -121 mEq/L. Between 12.1 -18H, 45 cases, with the Chloride concentration ranging from 76 -136.5 mEq/L. And in 18.1 – 24 H TSD the Chloride concentration is between 89.4 -124 mEq/L.

The same is depicted in the bar chart below.



Statistical analysis

Comparison	p value for Cl-
1 and 2	1.000
1 and 3	1.000
1 and 4	1.000
2 and 3	1.000
2 and 4	1.000
3 and 4	0.680

From the statistical analysis table above it is evident that there is no significant change in the concentration of Chloride in vitreous humor with increase in time since death.

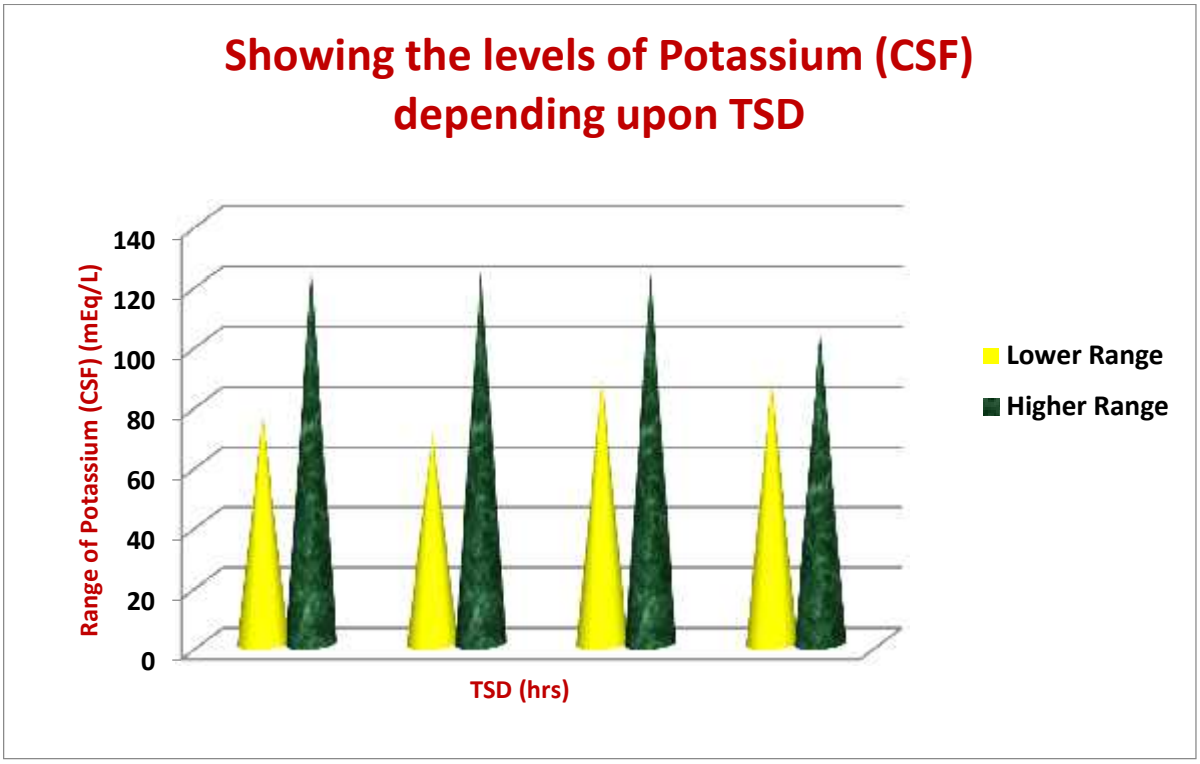
Table No.8

Showing the level of Potassium (mean & range) in CSF depending upon TSD

S.No.	TSD (hrs)	No. of Cases	Range of Potassium (mEq/L)	Mean (+) SD
1.	0 – 6.0	3	22 – 33.5	25.83 ±6.63
2.	6.1 – 12.0	29	16.9 – 30.4	27.23±3.29
3.	12.1 – 18.0	45	16 – 35.4	26.93±6.24
4.	18.1 – 24.0	23	26.8 – 31.6	29.60±1.40
	TOTAL	100		

The table above shows the distribution of the values of potassium concentration in CSF divided into 4 groups as per TSD. The four groups are: 0- 6 H, 6.1 – 12 H, 12.1 –18 H, and 18.1–24 H. In TSD of 0- 6 H there were 3 cases with the potassium concentration ranging from 22- 33.5 mEq/L. In TSD of 6.1 -12 H there were 29 cases with the potassium concentration ranging from 16.9 – 30.5mEq/L. Between 12.1 -18H there were 45 cases with the potassium concentration ranging from 16.5 -35.6 mEq/L. And in 18.1 – 24 H there were 23 cases with potassium concentration ranging between 26.8 to 31.6 mEq/L.

The same is depicted in the bar chart below.



Statistical analysis

Comparison	p value for K+
1 and 2	1.000
1 and 3	1.000
1 and 4	1.000
2 and 3	1.000
2 and 4	1.000
3 and 4	0.201

From the above Statistical analysis table it is evident that there is a no remarkable change in the levels of potassium concentration in the CSF with increasing time since death.

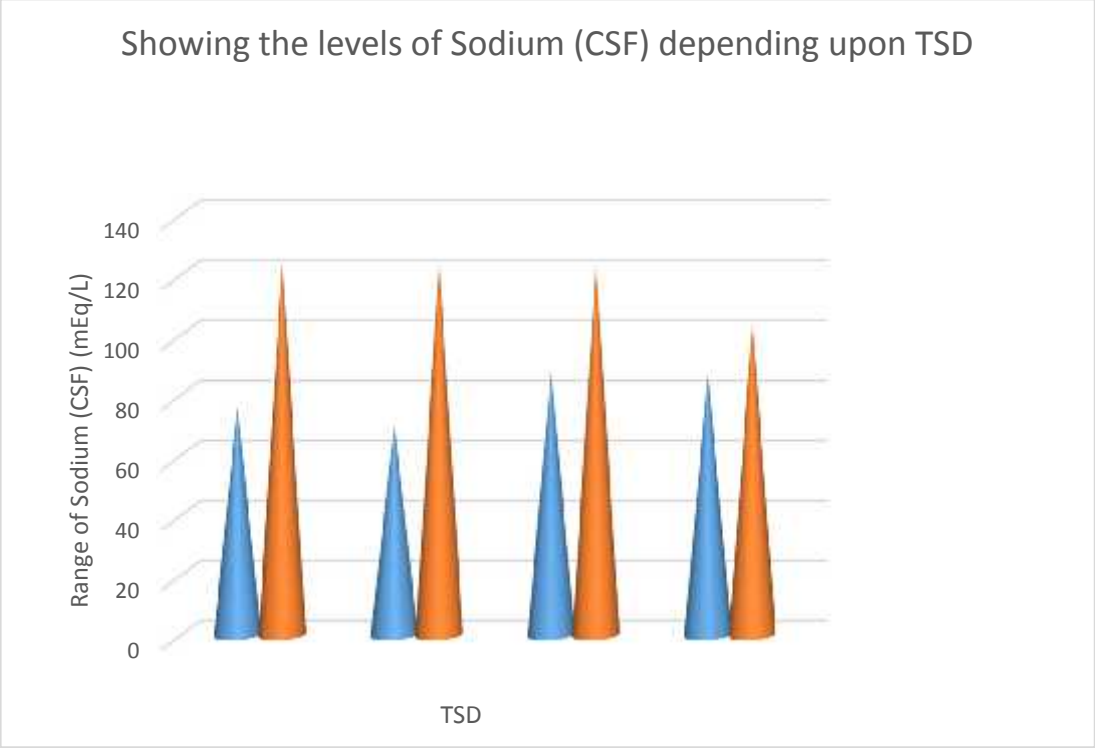
Table No.9

Showing the level of Sodium (mean & range) in CSF depending upon TSD

S.No.	TSD (hrs)	No. of Cases	Range of Sodium (mEq/L)	Mean (+) SD
1.	0 – 6.0	3	126 – 153	144±15.58
2.	6.1 – 12.0	29	110 – 144	117.89±7.59
3.	12.1 – 18.0	45	107 – 133.1	117.11±17.29
4.	18.1 – 24.0	23	110 – 129.6	117.27±7.27
	TOTAL	100		

The table above shows the distribution of the values of sodium concentration in CSF divided into 4 groups as per TSD. The value in four groups are: In 0- 6 H there are 3 cases, with the sodium concentration ranging from 126 – 153 mEq/L. In 6.1 -12 H there are 29 cases, with the sodium concentration varying from 110 -144 mEq/L. Between 12.1 -18H there are 45 cases, with the sodium concentration ranging from 107 - 133.1 mEq/L. And in 18.1 – 24 H, there are 23 cases, the sodium concentration is between 110 – 129.6 mEq/L.

The same is depicted in the bar chart below.



Statistical analysis

Comparison	p value for Na
1 and 2	0.009
1 and 3	0.005
1 and 4	0.008
2 and 3	1.000
2 and 4	1.000
3 and 4	1.000

From this statistical analysis table above it is evident that there is no significant change in the concentration of sodium in CSF with increase in time since death.

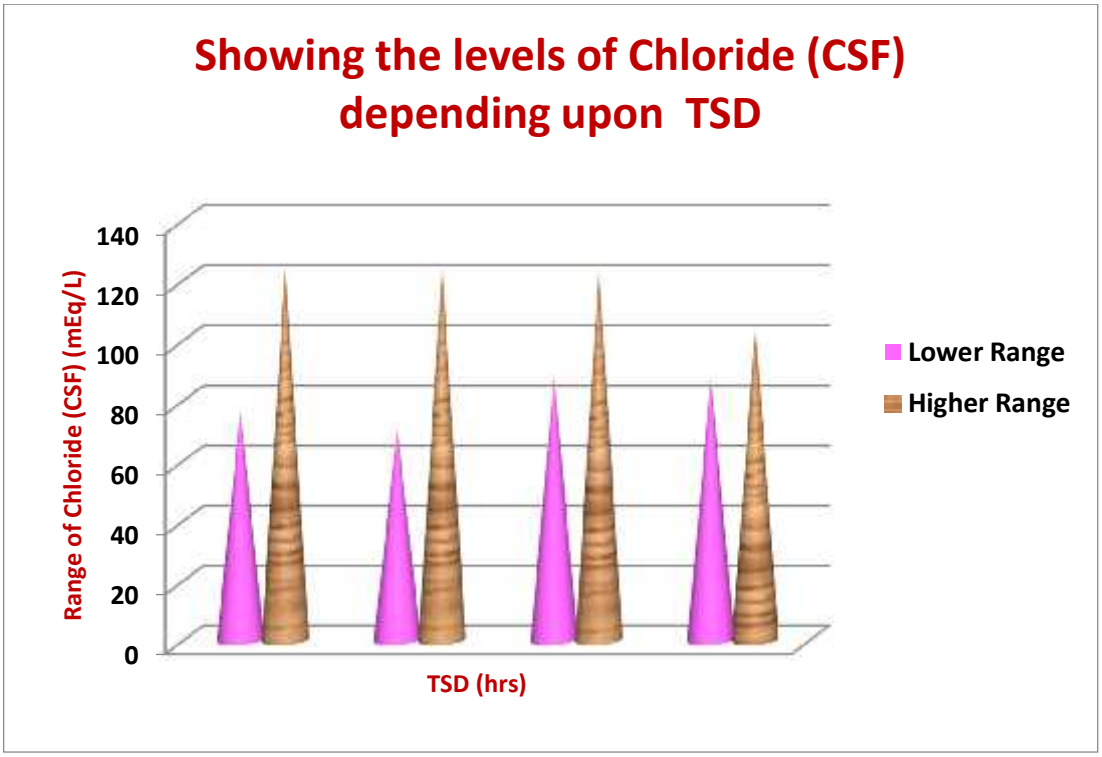
Table No 10

Showing the level of Chloride (mean & range) in CSF depending upon TSD

S.No.	TSD (hrs)	No. of Cases	Range of Chloride (mEq/L)	Mean (±) SD
1.	0 – 6.0	3	106 – 127	113±12.12
2.	6.1 – 12.0	29	95 – 119.2	108.74±7.68
3.	12.1 – 18.0	45	94 – 136.4	112.31±11.47
4.	18.1 – 24.0	23	95 – 138	111.84±15.47
	TOTAL	100		

The table above shows the distribution of the values of Chloride concentration in CSF divided into 4 groups as per TSD. The four groups are: 0- 6 H, 6.1 –12 H, 12.1 –18 H, and 18.1–24 H. In 0- 6 H there are 3 cases with the chloride concentration ranging from 106 -127 mEq/L. In 6.1 -12 H there are 29 cases with the chloride concentration ranging from 95 -119.2 mEq/L. Between 12.1 -18H there are 45 cases with the chloride concentration ranging from 94 -136.4 Eq/L. And in 18.1 – 24 H the chloride concentration is between 95 -138 mEq/L.

The same is depicted in the bar chart below.



Statistical analysis

Comparison	p value for Cl
1 and 2	1.000
1 and 3	1.000
1 and 4	1.000
2 and 3	1.000
2 and 4	1.000
3 and 4	1.000

From the statistical analysis table above it is evident that there is no significant alteration in the concentration of Chloride in CSF, with increase in time since death.

No. of cases in various age group according to TSD

TSD	20 -29	30-39	40-49	50-59	60 -70	TOTAL
0-6 H	-	2	-	-	1	3
6.1-12 H	6	6	8	4	5	29
12.1-18 H	14	9	3	11	8	45
18.1 -24 H	4	4	5	5	5	23

The table shows the distribution of cases in the various age group and as per their TSD. The samples were grouped into 5 age groups i.e 20 – 29 yrs, 30 – 39 yrs, 40 – 49 yrs, 50 – 59 yrs and 60 - 70 yrs and TSD divided into 4 groups as up to 6 H, 6.1 to 12 H, 12.1 to 18.1 H and up to 24 H. In the TSD of up to 6 H there were 3 cases. In TSD of 6.1 to 12 H there were 29 cases, in TSD 12.1 to 18 H there were 45 cases, and in TSD 18.1 to 24 H there were 23 cases.

Range of electrolytes in TSD of 0-6 H:

Sl.No	AGE GP	No.of cases	K+ (VH)	Cl -(VH)	K+ (CSF)	Cl- (CSF)
1.	20 -29	-	-	-	-	-
2	30 -39	2	8.2	121	22	106
3.	40 -49	-	-	-	-	-
4.	50 -59	-	-	-	-	-
5.	60-7 0	1	8.3	106	33.5	127

The table above shows the concentration of Potassium and Chloride in VH and CSF in the various age group at TSD of up to 6 H, irrespective of the cause of death. In TSD 0- 6 H there were only 3 cases, i.e 2 cases in the age group of 30 – 39 yrs and 1 case in 60 -70 yrs.

The table below shows the range of potassium and chloride in VH and CSF in the TSD of 0-6 H irrespective of the age group.

Range of electrolytes in TSD of 0 – 6 H irrespective of the age group :

Sl.No	Electrolytes	Range
1.	VH (K+)	8.2 – 8.3
2.	VH (Cl--)	106 -121
3.	CSF (K+)	22 – 33. 5
4.	CSF (Cl-)	106 - 127

Range of electrolytes in TSD of 6.1- 12 H

Sl.No	AGE GP	No.of cases	K+ (VH)	Cl- (VH)	K+(CSF)	Cl-(CSF)
1.	20 -29	6	7.2 - 10.6	79 - 121	16.9 – 29.4	101.2 – 117.4
2.	30 -39	6	7.9 - 12	61 - 123	20 - 31	95.4 – 119.7
3.	40 -49	8	6.3 - 10	88 - 122	22.6 - 30	95 – 119.2
4.	50 - 59	4	6.9- 12.5	87 - 120	26.4 - 30.4	108.6 – 109.4
5.	60-70	5	7.3 – 13.5	94 - 116	26 -30.6	109 – 117.8

The table above shows the concentration of Potassium and Chloride in VH and in CSF in the various age group at TSD of 6.1 hrs to 12 H, irrespective of the cause of death. In that group there were 29 cases with 6 cases each in the age groups of 30 – 29 yrs, and 30 – 39 yrs and 8 cases in age group of 40 – 49 yrs,4 cases in age group of 50 – 59 yrs and in 60 - 70 yrs there were 5 cases.

The table below shows the range of potassium and Chloride in VH and CSF in the TSD of 6.1 to 12 H irrespective of the age group.

Range of electrolytes in TSD of 6.1 -12 H irrespective of the age group :

Sl.No	Electrolytes	Range
1.	VH (K+)	6.3 - 13.5
2.	VH (Cl-)	61 - 123
3.	CSF (K+)	16.9 - 31.0
4.	CSF (Cl-)	95 – 119.7

Range of electrolytes in TSD of 12.1 -18 H :

AGE GP	No. of cases	K+(VH)	Cl- (VH)	K+(CSF)	Cl-(CSF)
20 -29	14	6.6 – 12.3	75 - 136	16 - 34	96 - 124
30 -39	9	8.1 -12.5	84 -124	16.5 -33	95 – 136.4
40 -49	3	6.3 – 11.5	88 - 122	26.1- 31.4	95.1 – 138
50 -59	11	6.9 - 12	76 -135	28.6 – 31.6	95.1 – 123.8
60 - 70	8	6.5 – 10.0	75.9 - 123	24 – 33.5	98 - 120

The table above shows the concentration of Potassium and Chloride in VH and in CSF in the various age group at TSD of 12.1 to 18 H irrespective of the cause of death. In TSD 12.1 -18 H there were totally 45 cases with 14 cases in the age group of 20 – 29 yrs, 9 cases in 30 – 39 yrs, 3 cases in age group of 40 – 49 yrs,11 cases in age group of 50 – 59 yrs and 3 cases 60 -70 yrs .

The table below shows the range of potassium and chloride in VH and CSF in the TSD of 12.1 to 18 H irrespective of the age group.

Range of electrolytes in TSD of 12.1 - 18 H inspite of any age group :

Sl.No	Electrolytes	Range
1.	VH (K +)	6.3 – 12.6
2.	VH (Cl-)	75 – 136
3.	CSF (K +)	16 - 33
4.	CSF (Cl-)	95 - 138

Range of electrolytes in TSD of 18.1 -24 H :

AGE GP	No. of cases	K+ (VH)	Cl- (VH)	K+ (CSF)	Cl- (CSF)
20 -29	4	8.3 – 13.5	98.7 123.5	29.8 -31.4	95.1 -124.4
30 -39	4	8.2 -12.5	121 - 124	28.4 – 31.2	95 - 126
40 -49	5	8.1 – 12.4	89 – 124.2	28.1 – 28.5	95 - 138
50 -59	5	7 – 13.5	87.5 - 124	28.6 – 31.6	95.1 – 123.8
60 -70	5	8.2 – 13.8	88 - 122	28.4 - 31	96 – 124.4

The table above shows the concentration of Potassium and Chloride in VH and in CSF in the various age group at TSD between 18.1 -24 H irrespective of the cause of death. In TSD 18.1 to 24 H there were totally 23 cases with 4 cases each in the age group of 20 – 29 yrs and 30 – 39 yrs, and 5 cases each in age group of 40 – 49 yrs,50 – 59 yrs and 60 - 70yrs.

The table below shows the range of potassium and chloride in VH and CSF in the TSD of 18.1 to 24 H irrespective of the age group.

Range of electrolytes in TSD of 18.1 - 24 H in spite of any age group :

Sl.No	Electrolytes	Range
1.	VH (K+)	7 – 13.5
2.	VH (Cl-)	87.5 – 124 .2
3.	CSF (K+)	28.1 – 31.6
4.	CSF (Cl -)	95 - 138

Case distribution due to varying COD as per TSD

TSD	POISON	RTA	HANGING	SNAKE BITE	OA	LIGHTNING	Total
0-6 H	1	2	-	-	-	-	3
6.1-12 H	14	8	5	1	1	-	29
12.1-18 H	26	14	4	-	-	-	45
18 -24 H	12	5	2	1	1	2	23

The table above shows number of cases with different causes of death divided as per their TSD, i.e up to 6 H there were only 3 cases, one of poison ,2 from RTA. In 6.1 to 12 H there were 29 cases, i.e 14 cases of poison, 8 from RTA, 5 from hanging and 1 each from snake bite and other accidents. In 12.1 to 18 H there were 45 cases, out of which 26 cases of poison,14 cases of RTA, and 4 cases of hanging and in 18.1 to 24 H there were 23 cases distributed as 12 from poison, 5 from RTA, 2 each from hanging and lightning and 1 case each from other accidents and snake bite.

Showing distribution of cases in death due to POISON and their electrolytes range in VH & CSF

TSD	No, of cases	K+(VH)	Cl- (VH)	K+ (CSF)	Cl- (CSF)
0 -6 H	1	8.3	106	32.5	127
6.1 – 12 H	14	6.5 -13.5	88 -120	28.5– 33.5	95 - 124
12.1 – 18 H	26	6.9 – 12.4	76 - 135	26.8– 35.4	106 - 120
18.1 – 24 H	12	8 – 11.8	88.3 -123.5	30.4 -36.5	121 - 124

The table above show the range of potassium and chloride in cases of death due to poison, divided as per their TSD. It is evident from the table that there is no remarkable variation in the concentration of electrolytes range in VH and CSF as per TSD.

Showing distribution of cases in death due to RTA and their electrolytes range in VH & CSF

TSD	No. of cases	K+(VH)	Cl- (VH)	K+ (CSF)	Cl- (CSF)
0 -6 H	2	8.2	121	22	106
6.1 – 12 H	8	6.9 – 10.1	79.2 -122	17 - 31	95.4 - 117
12.1 – 18 H	14	7.6 – 12.3	80.3 - 123	16 – 34.4	95 - 116
18.1 – 24 H	5	8.4 – 13.5	98.7 - 124	13.8 – 36.8	95 - 124

The table above show the range of potassium and chloride in VH & CSF in cases of death due to RTA, divided as per their TSD. It is evident from the table that the concentration of VH and CSF electrolytes show no remarkable change in electrolytes range as per TSD.

Showing distribution of cases in death due to HANGING and their electrolytes range in VH and CSF

TSD	No.of cases	VH (K+)	VH (Cl-)	CSF(K+)	CSF (Cl-)
0 -6 H	-	-	-	-	-
6.1 – 12 H	5	6.3 - 12	79.2 – 122	27.2 – 29.7	108.7 - 118
12.1 – 18 H	4	6.9 – 9.1	87.8 – 122	21 – 31.8	101 – 121.3
18.1 – 24 H	2	8.3 – 10.2	120 – 120.6	28.3 – 29.7	95.1 - 96

The table above show the range of potassium and chloride in VH & CSF in cases of death due to hanging, divided as per their TSD. It is evident from the table that there is no significant variation in electrolytes range in VH and CSF as per TSD.

Range of VH (K+) in varied TSD

Sl.No	TSD (hrs)	Range of VH (K+)
1.	0 – 6	8.2 – 8.3
2.	6.1 – 12	6.3 – 13.5
3.	12.1 – 18	6.3 – 12.6
4.	18.1 - 24	7 – 13.5

The table shows the range of VH potassium in the TSD of 0- 6 H as 8.2 – 8.3. In 6.1 – 12 H from 6.3 – 13.5, in 12.1 – 18 H from 6.3 – 12.6,and 18.1 -24 H from 7 – 13.5. From the above table it is evident that value does not change significantly with time.

Range of VH (Cl-) in varied TSD

Sl.No	TSD (Hrs)	Range of VH Cl-)
1.	0 – 6	106 - 121
2.	6.1 – 12	61 - 123
3.	12.1 – 18	75 – 136
4.	18.1 - 24	87.5 – 124

The table shows the range of VH Chloride in the TSD of 0- 6 H as 106 - 121. In 6.1 – 12 H from 61 – 123, in 12.1 – 18 H from 75 to 136 ,and 18.1 -24 H from87.5 -124 . From the above table it appears that the value is not significantly variable with time.

Range of CSF (K+) in varied TSD

Sl.No	TSD (Hrs)	Range of CSF (K)
1.	0 – 6	22 – 33.5
2.	6.1 – 12	16.9 – 31
3.	12.1 – 18	16 - 33
4.	19.1 - 24	28.1 – 31.6

The table shows the range of CSF potassium in the TSD of 0- 6 H as 22 – 33.5 . In TSD of 6.1 – 12 H potassium ranges from 16.5 - 31, in TSD from 12.1 – 18 H potassium varies from 16 – 23 ,and in TSD from 18.1 -24 H the value varies from 28.1 to 31.6. From the table it is evident that the range does not alter significantly with time.

Range of CSF (Cl) in varied TSD

Sl.No	TSD (Hrs)	Range of CSF (Cl)
1.	0 – 6	106 – 127
2.	6.1 – 12	95 – 119.7
3.	12.1 – 18	95 - 138
4.	18.1 - 24	95 - 126

The table shows the range of CSF Chloride in the TSD of 0- 6 H as 106 - 121. In TSD of 6.1 – 12 H the value of chloride is from 95 to 119.7, in TSD 12.1 – 18 H the value varies from 95 – 138, and from 18.1 -24 H the value ranges from 95 - 126. From the above table it is evident that the value does not alter with time change.

DISSCUSION

In medico legal autopsies knowledge of accurate estimation of TSD is very essential. Apart from the use of other physical methods like changes in eye, cooling of body, postmortem lividity, rigor mortis, putrefaction and decomposition changes, chemical changes is also being used now.

TSD is calculated using these physical and chemical changes but these changes are never constant and mostly variable. Determination of PMI also becomes difficult due the various exogenous and endogenous factors acting on them. Despite an extensive literature on this, the determination remains difficult. The dogmatic application of rules and formulae based on single and isolated observations is a guarantee of inaccuracy.

Various biochemical changes occur in the body just after death and progress in fairly uniform manner. The various fluids used to determine PMI are blood, serum, CSF, Aqueous & Vitreous humor present in vitreous body. Vitreous is a transparent gelatinous substances that fills the posterior chamber. Its commonly used to determine TSD as it is located in a safe anatomical position ,mostly undisturbed and unaltered. It is also preferred as it is easy to obtain in sufficient quantity with no contamination and without affecting the

eye cosmetically. The vitreous humor is relatively inert and usually not influenced by fluctuations in blood chemistry of the human body

Most of the studies in the past admit the values of vitreous humour potassium as a valuable marker in the determination of PMI, it raises considerably with increasing postmortem interval. But other electrolytes such as sodium and chloride show very variable change and is of limited use in estimating time of death.

As per TABLE 1, my samples of 100 cases were divided into male and female cases. Out of which 70 cases from male and 30 cases from females.

In TABLE -2 the samples were divided into 5 groups as per their age. That is between 20 to 29 years consisted of 23% of cases, 21% of cases in 30-39 years,17% of cases in age group of 40 – 49 years ,19% of cases in 50- 59 years and 60 - 70 years there were 20% of cases .The table further subdivides the samples into male and female categories.

Table 3 & 4 shows the percentage distribution of cases as per TSD. The total TSD is grouped into 4 ,such as 0-6 hrs , 6.1-12 hrs, 12.1 -18 hrs and 18.1 -24 hrs. The number of cases in each group were 4, 29, 45,and 22 respectively.

Table 5,6,7, 8,9 and10 shows the distribution of values of potassium, Sodium and Chloride in VH and CSF respectively as per their TSD irrespective of their age and cause of death.

As my study involves assessing TSD, the samples were divided into 5 , age groups and TSD into 4 groups as mentioned above , and the cases were grouped as per the TSD. Which consisted of 3 cases in TSD of 0 to 6 H, 29 cases in the group of TSD from 6.1 to 12 H, 45 cases in the TSD of 12.1 to 18 H and 23 cases in the group of 18.1 to 24 H as shown in table 11.

In table 12, 14, 16 and 18 I have shown the concentration of electrolytes in VH and CSF in the time duration of TSD of 0-6 H, 6.1 to 12 H, 12.1 to 18 H and 18.1 to 24 H respectively divided as per the age group. Table 13, 15, 17 and 19 shows the range of electrolytes in the TSD of 0-6 H, 6.1 to 12 H,12.1 to 18H and 18.1 to 24 H respectively.

Table- 20 shows the distribution of cases according to the varying cause of death divided as per TSD in to 4 groups. In my study of 100 sample. 53 cases were of poison , 29 cases of RTA ,11 cases of hanging and 2 cases each of snake bite , other accidents and lightning.

In table 21 I have shown the distribution of cases of death due to poison as per their TSD. The table also represents the value of electrolytes as per TSD.

Similarly in table 22 and 23, I have shown the distribution of cases in death due to RTA and HANGING as per TSD. The table shows the value of electrolytes' in VH and CSF as per the TSD.

In table 24,25,26 and 27 I have summarized the value in ranges of electrolytes in VH and CSF as per the TSD irrespective of the cause of death.

The table below summarizes my values of electrolytes as per TSD irrespective of the age group and cause of death. This table also represents the summary of all the tables from 21 to27.

Electrolyte range in varied TSD

Sl.No	TSD(Hrs)	K+(VH)	Cl-(VH)	K+(CSF)	Cl-(CSF)
1	0- 6	8.2 – 8.3	106 - 121	22 – 33.5	106 - 127
2	6.1 – 12	6.3 – 13.5	61 -123	16.9 -31.0	95 – 119.7
3	12.1 – 18	6.3 – 12.6	75 - 136	16 – 33.0	95 - 138
4	18.1 – 24	7 – 13.5	87.5 -124	28.1 – 23.6	95 - 126

The effect of level of rise of electrolytes in vitreous and CSF in both gender, as Tabulated in 1 was analysed. The analysis does not show any

significant change in the levels of vitreous and CSF electrolytes in male and female cases. The observation is also authenticated by other workers.

Analysis based on age, not show appreciable role in changes in the level of concentration in vitreous humour after death.

Analysis of cases on the value of electrolytes depending on the cause of death as per TSD did not show any reasonable variation in potassium and chloride value of VH and CSF.

In my study of assessing time since death using electrolytes from VH and CSF, in over 100 samples, all the value of electrolytes falls in the same range with no obvious difference with change in TSD irrespective of age, gender and cause of death.

Hence assessing time since death using electrolytes from VH and CSF is not of much use if accuracy is in need.

CONCLUSION

These are the conclusion from my study:

1. The changes in electrolytes (sodium, potassium and chloride) in VH and CSF do not show any significant relation with the sex (male or female) of the individual.
2. The changes in electrolytes (sodium, potassium and chloride) in VH and CSF do not show any significant relation with the age of the individual.
3. The changes in electrolytes (sodium, potassium and chloride) in VH and CSF do not show any significant change with the various cause of death of the individuals.
4. The TSD and value of electrolytes do show any particular relationship in estimating time since death.

RECOMMENDATIONS

1. The study should be conducted in huge samples.
2. The study should be conducted in varied regions to find the relation of TSD with change in electrolytes.

ANNEXURE – 1: PROFORMA

ASSESSING TIME SINCE DEATH BY USING CHANGES IN ELECTROLYTES IN C.S.F AND VITREOUS HUMOUR IN BODIES SUBJECTED TO AUTOPSY

1. Sl. No./Case No Date

2. Police station & Crime no.:

3. Name :

4. Age from case record/ Police record:

5. Sex (M/F) :

6. Address :

7. Occupation :

8. Married /Unmarried :

9. Physical Examination :

Nourishment : (poor/moderate/well)

Built : (poor / moderate /well)

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SL No.	NAME	AGE	SEX	DIAGNOSIS	PM.NO	DOD	TOD	DOS		CSF			V.H			RM	TSD	2.58 x k-9.3	FORM FR POT
										Na	k	Cl	Na	k	Cl			V.H	csf
1	SELVI	27	F	HANGING	688/16	13.05.16	5:00 AM	13.05.16	14:00 PM	111.8	29.8	95.1	153	8.3	120	+	21 H	12.1	18.7
2	ARUMUGAM	50	M	RTA	692/16	13.05.2016	5.30 AM	13.05.16	14.30 PM	113.1	30.2	95.1	149	7	118	+	21 H	8.76	19.15
3	GANAPATHY	45	M	POISON	714/16	18.5.16	4.30 AM	18.5.16	13 .30 PM	110.9	29.4	95.6	146	9.9	88.8	+	9 H	15	18.3
4	RAJASINGH	51	M	OA	716/16	18.5.16	16.30pm	18.5.16	14 : 00 PM	112.8	29.6	95.1	141	10.6	87.2	+	22 H	18	18.6
5	MUNIYANDI	55	M	POISON	720/16	19.5.16	21.30 pm	19.5.16	15.10 PM	112	25.9	96.2	142	9.8	88.1	+	16 h	15.2	14.8
6	SHANTHAKUMARA	32	F	RTA	738/16	24.5.16	5.15am	19.5.16	15.30 PM	113	31	95.4	130	10.1	87.6	+	10 H	16.7	19.94
7	SHANTHA	55	F	RTA	739/16	24.5.16	2.25pm	24.5.16	15.45 PM	110	34.5	94.9	138	10.6	89.2	+	13 h 20	18	23.44
8	NANDHINI	20	F	POISON	741/16	25.5.16	23 pm	26.5.16	14 : 00 PM	111	34	96	141	10.5	88	+	15 h	16.9	24
9	CHANDRALEKHA	43	F	POISON	1520/16	03.11.16	9.30 PM	4.11.16	11:00 AM	112	29.4	95	147	10	88	+	8 H 30	16.5	19.02
10	SHANMUGAM	29	M	POISON	1522/16	03.11.16	3.20 AM	3.11.16	13:00 PM	123	27	113.8	137	9	118	+	9 H40	13.92	15.9
11	NALLATHAI	57	F	RTA	1530/16	03.11.16	2.30 AM	3.11.16	15 :15 PM	112	30	95.8	132	7.6	87.5	+	12 H 45	10.3	18.9
12	KANNAN	30	M	RTA	1529/16	3.11.16	9.30 AM	3.11.16	15 .30 PM	153	22	106	171	8.2	121	+	6 H	11.85	10.9
13	MARRIAPPAN	25	M	POISON	152/17	8.2.17	3.15 AM	8.2.17	14 :00PM	124	27.4	114.4	138	9.1	118.9	+	10h 45	14.17	15.7
14	ANNATHAMMAL	65	F	RTA	153/17	8.2.17	7.10 AM	8.2.17	14:20 PM	111.2	30.2	104.9	134	7.3	104.9	+	7 H 10	9.53	19.15
15	MUTHUSWAMY	40	M	FALL	154/17	8.2.16	6.45 AM	8.2.17	14 : 00PM	110	30	104	133	7	103.9	+	7 H15	8.76	18.9
16	SUBBAIAH	57	M	POISON	157/17	8.2.17	9 :00PM	9.2.17	13 ::00 PM	107	17	101	137	12	135	+	16 H	21.66	5.94
17	MALLIKA	21	F	POISON	159/17	9.2.17	11:00 PM	10.2.17	14.30 PM	106.8	16	101.2	136	10.6	89.5	+	15h30	18	5.61
18	MARIMUTHU	34	M	POISON	164/17	9.2.17	10:00 PM	12.2.17	14 :30 PM	106	16.5	101	136.5	10.5	88.3	+	16 H30	16.9	5.68
19	KARUPUSWAMY	48	M	POISON	174/17	13.2.17	2:00 PM	14.2/17	13 00 PM	112	28.2	95	8. 2	10.7	89	+	23 H	18.3	19.34
20	MURUGAN	43	M	POISON	175/17	13.2.17	6.00PM	14.2.17	13: 00 PM	111	28.5	95.4	152	8.1	119	+	20 H	11.59	17.44
21	CHANDRA	32	F	POISON	177/17	13.2.17	6:00 PM	14.2.17	13.30 PM	110	29	95	151	8.2	121	+	20 h 30	11.85	17.94
22	CHOCKALINGAM	61	M	POISON	179/17	14.2.17	11:00 AM	14.2.17	15.20 PM	126	33.5	127	144	8.3	106	+	4 H20	12.11	22.44
23	PARTHIBEN	22	M	RTA	181/17	14.2.17	23.50 PM	15.2.17	14 :00 PM	105.8	16	101	135	10.5	88	+	14 H10	17.7	5.61
24	RAMESH	27	M	RTA	183/17	14.2.17	20 :40 PM	15.2.17	13.30 PM	108	17.3	101.7	139.5	12.3	136.3	+	17 h10	22.4	6.1
25	JEYAPAUL	23	M	RTA	184/17	15.2.17	7.20 AM	15.2.17	14 :00 PM	117	16.9	117.7	165	8.5	104	+	6h 40	12.6	5.64
26	PETCHIAMMAL	60	M	SNAKE BITE	185/17	15.2.17	2:00 AM	15.2.17	14.30 PM	128	26	109.3	124	8.3	94.1	+	11h 30	12.11	14.94
27	MUTHURAJ	30	M	POISON	188/17	14.2.17	10.30 PM	15.2.17	15.20 PM	125	33	126	142	8.6	109	+	15 h 10	12.88	15.1
28	RAJA	70	M	POISON	202/17	18.2.17	00:10 AM	19.2.17	14:!0 PM	124	33.5	102	141.5	10	90		14 H10	16.5	15.7
29	SAPANI	70	M	POISON	206/17	20.2.17	23:20 PM	20.2.17	15.30 PM	123	33.8	98	142	9.5	89		16 H 30	15.21	22.74
30	UMARKATHAB	55	M	POISON	209/17	20.2.17	20 :1 5PM	21.2.17	15 :10 PM	110.4	28.9	95.8	150.6	8.1	120		19 H 10	11.59	17.55
31	TAMILARASI	40	M	POISON	214/17	21.2.17	7.12 Am	21.2.17	13.20 PM	128	27.1	105	150.6	8.1	104.4		7 h	11.59	15.9
32	KARRUPUSWAMY	35	M	POISON	215/17	21.3.17	23 .10 PM	22.2.17	13 :00 PM	118	35.4	101.6	137	11.2	87.8		13 H 50	19.5	24.34
33	MUTHURAJ	35	F	POISON	217/17	21.3.17	23:00 PM	22.2.17	15.20 PM	122	32.8	98.5	141	9.6	114.7		16 h 20	15.46	21.74
34	MARI	67	F	HANGING	222/17	23.2.17	15:00 PM	24.2.17	12:05 PM	128	28.3	96	141	10.2	120.6		21 H	17.01	17.12
35	RAMU	40	M	POISON	244/17	27 .2.17	14:00 PM	28.2.17	13.15 PM	122.6	28.1	138	122	8.6	124.2		23H 45	12.88	16.84
36	ESAKIMUTU	46	F	RTA	245/17	27.2.17	20:00 PM	28.2.17	13.20 PM	110	27.9	136	120.4	10.9	88.6		17H 20	18.8	16.91
37	AJEELA	27	F	RDO	275/17	8.3.17	1:oo AM	8.3.17	16::00 PM	127.6	27	124	141.4	7.9	113.4		15 H	11.08	15.94
38	ANNAMALAI	70	M	RTA	276/17	8.3.17	1.30 AM	8.3.17	16.30 PM	126.2	27.2	120	158	7.9	123		15H 30	11.08	15.89
39	SUBRAMANIUM	48	M	RTA	278/17	8.3.17	16.45 PM	9.3.17	13: 00 PM	110.7	28.4	95.3	150.7	8.4	119.4		20 H 15	12.37	16.78
40	ARULRAJ	48	M	POISON	279/17	8.3.17	20.30 PM	9.3.17	13.30 PM	117.6	27.4	136.4	141.2	10.4	88.4		17 H	17.53	16.3
41	SHANMUGAVEL	38	M	POISON	280/17	8.3.17	20.30PM	9.3.17	15.30 PM	110.5	28.4	124	149.7	8.6	120		19 H	12.88	16.78
42	SHANKARALINGAM	65	M	LIGHNING	281/17	8.3.17	18.30 PM	9.3.17	14.00 PM	112	31.4	124.3	125	12.7	103		19 H.30	23.46	20.34
43	ATTASWAMY	53	M	HANGING	283/17	8.3.17	22:00 PM	9.3.17	14 30 PM	122.6	31.8	121	136	8.4	115		16 H30	12.34	20.74
44	VEMBHU	70	M	POISON	284/17	8.3.17	21 :00 PM	9.3.17	16::00 PM	111.3	28.4	96.7	136	8.2	98.3		19 H	11.85	16.78
45	PARAMAJOTHY	70	M	HANGING	287/17	10.3.17	3:00 AM	10.3.17	14 : 00 PM	113.4	26.7	109.4	123.5	8.2	117.6		11 H	11	15.64
46	GOPALASWAMY	30	M	RTA	294/17	12.3.17	6.15 AM	12.3.17	15 .45 PM	114.2	27.2	108.6	123.8	7.9	117		10 H	11	15.89
47	KRISHNASWAMY	60	M	POISON	302/17	14.3.17	3.45 AM	14.3.17	14 : 00 PM	112.8	27.6	109.8	122	8	118.2		10H 15	11.34	16.27
48	PERIYASWAMY	38	M	RTA	310/17	15.3.17	21:00 PM	16.3.17	13:00 PM	118.4	27.8	124.2	146	10.8	80.3		15 H	18.4	16.74
49	RASAIAH	65	M	POISON	314/17	16.3.17	17.15 PM	17.3.17	14 .20 PM	127.5	28.4	96.4	141.6	12.8	88.2		21 H	23.72	16.78
50	RAJALINGAM	48	M	HANGING	317/17	17.3.17	7.00AM	17.3.17	13.30 PM	112	28.8	117.2	141	6.3	103.7		6H 30	6.95	16.75

51	VELAMMAL	51	M	POISON	319/17	17.3.17	4.10 AM	17.3.17	14.20 PM	114.5	28.6	109.4	132	7.1	117.5		10 H 10	9.01	16.7
52	ARUNACHALUM	58	M	POISON	334/17	21.3.17	1:00 AM	21.3.17	14:20 PM	113.4	26.4	108.7	133.1	6.9	116.9		11 H20	8.5	15.3
53	CHELLAPA	65	M	POISON	337/17	21.3.17	1:00 AM	21.3.17	13:30 PM	122	24	106.9	134	6.5	75.9		12 H30	7.47	12.9
54	RAJASEKAR	30	M	RTA	342/17	22/3/17	8:00 AM	22.3.17	14:00 PM	144.5	20	102	155	9.8	61.9		8 H	15.98	8.94
55	SUDALAI	27	F	HANGING	345/17	23.3.17	1.30 AM	23.3.17	14.10 PM	145	21	101	139	6.9	122		12 H40	8.5	9.94
56	SUVELU	56	F	HANGING	346/17	23.3.17	2.30 AM	23.3.17	16.10 PM	117.6	27.6	119.6	140	7 .2	120.4		14H30	9.08	16.2
57	SIVAKUMAR	21	M	POISON	349/17	24.3.17	2.25 AM	24.3.17	14.45PM	122.4	24	106.9	136.4	6.6	76		12H 45	7.78	12.9
58	SIVAJI	21	M	POISON	350/17	24.3.17	5:00 AM	24.3.17	13.45 PM	121.5	20.8	102.7	138.6	10.6	103		8 H 45	18	9.74
59	MUPIDATI	60	F	RTA	354/17	26.3.17	23.20 PM	27/3/17	13.30 PM	121.1	26	103.4	132	8.1	119.5		14 H10	11.5	14.9
60	SUDALAI	35	M	RTA	359/17	26.3.17	20.45 PM	27.3.17	12.30 PM	121.8	31.5	123.8	132.2	8.1	118.8		15 H 15	11.5	20.33
61	RAJA	21	M	RTA	360/17	26.3.17	17:40 PM	27.3.17	13.30 PM	110.9	30.8	124.1	141	13.5	98.7		19 H50	25.5	19.7
62	JEYAPAUL	54	M	POISON	362/17	26.3.17	3.15 AM	27/3/17	13 :45 PM	114.6	28.6	109.2	141	13.5	87.8		10 H30	25.5	17.54
63	SUNDAR	25	M	RTA	364/17	27.3.17	6.30 AM	27.3.17	14 :40 PM	112.6	27.6	101.2	142.6	8.8	79.2		6 H 10	13.4	16.7
64	MAKARASI	29	F	POISON	367/17	26.3.17	21.50 PM	27.3/17	16.10 PM	112.5	26.8	123.9	142.6	8.87	101		18 H20	13.4	15.74
65	SOUNDARAJAN	25	M	RTA	373/17	29.3.17	23.45 PM	30.3.17	13:00 PM	121	16.2	106.4	130.8	9.9	88.4		13H 45	16.24	10.5
66	GOMMU	55	M	LIGHTNING	375/17	29.3.17	16 .30 PM	30.3.17	13 :00 PM	112.8	31.6	123.8	139.2	12.4	98.7		20 H30	22.69	20.5
67	SHAHULHAMEED	50	M	POISON	387/17	2.4.17	1.00 AM	02.04.17	15.30 PM	121.4	15.8	106.8	131.2	6.9	76		12 H 3O	8.5	12.2
68	SENTHIL RAJA	33	M	HANGING	396/17	3.4.17	5:00 AM	3.4.17	14.50 PM	114.5	29.7	108.7	122.9	9.4	86.8		9 H 50	14.9	18.64
69	MUTHUSELVI	20	F	HANGING	399/17	3.4.17	3:00 AM	3.4.17	15 PM	122.1	29.4	109	131.4	7.2	121.2		12 H	9.27	18.3
70	RASAIAH	55	M	POISON	400/17	3.4.17	0.45 AM	3.4.17	13.15PM	121.9	30.4	108.6	136.2	8.2	120.9		11 H30	11.6	19.34
71	MAHALAKSHMI	35	F	POISON	402/17	3.4.17	1:00 AM	3.4.17	13.30 PM	121.6	30.2	107.9	136.4	8.6	120.4		13 H 30	13.1	19.33
72	RAJESH	32	F	RTA	403/17	4.4.17	3 .00 AM	4.4.17	12.30 PM	122.6	27.6	112.2	133.1	8.9	84.3		14 H30	13.66	16.2
73	PITCHAMMAL	35	F	POISON	404/17	04.04.17	1.15 AM	4.4.17	12.45 PM	121.9	27.1	118.4	133.1	8.8	120.5		12H10	13.45	16.7
74	KARTHIKA	28	F	POISON	405/17	5.4.17	2:00 AM	5.4.17	14.45PM	122.6	27.4	118.8	133.9	9.1	121.6		12 H45	14.17	16.04
75	SUBRAMANIUM	47	M	POISON	407/17	5.4.17	3:00 AM	5.4.17	15.OO PM	121.8	27.3	119.2	132.5	9.2	117.6		12 H	14.32	16.34
76	SHANMUGAM	70	M	POISON	408/17	5.4.17	1.30 AM	5.4.17	14.45PM	121.4	26.8	120.6	133.4	9.7	118		13 H30	15.72	16.1
77	SUBRAMANI	45	M	POISON	410/17	6.4.17	5.30 AM	6.4.17	14.30 PM	113.9	22.6	117.4	121.9	8.8	103.2		9 H	13.45	15.74
78	PERIYATHAI	55	F	POISON	411/17	5.4.17	21.45 PM	6.4.17	14 :00 PM	121.6	31.2	123.4	141.3	7.9	97.3		16 H15	11.08	20.3
79	MURUGAN	55	M	POISON	412/17	6.4.17	5.30 AM	6.4.17	15.20 PM	13.4	31.6	124.6	139.6	11.2	102		17H50	19.59	20.22
80	RAJU	21	M	RTA	413/17	5.4.17	23.30 PM	6.4.17	14.30 PM	122.8	30.9	119.5	132.8	10.8	87.5		15 H	18.5	20.34
81	ESALAAMMAL	69	M	POISON	414/17	6.4.17	1 :00AM	6.4.17	14.20 PM	123.4	29.8	117.8	135.7	10.2	120.8		13 H20	17.01	19.8
82	SANKARAN	44	M	POISON	417/17	7.4.17	1:00 AM	7.4.17	13 PM	123.3	29.5	118	131.1	8.3	122		12 H	12.11	19.9
83	MARIMUTHU	25	M	HANGING	423/17	8.4.17	22 :20 PM	9.3.17	14:00 PM	133.1	31.4	121.3	132.6	9.1	87.8		15 H40	14.17	20.3
84	CHELLADURAI	26	M	RTA	430/17	10.4.17	22.20 PM	11.4.17	14:00 PM	123.4	26.8	120.8	132.6	8.7	121.8		13 H 40	13.33	15.74
85	MARAGATHAM	66	F	RTA	431/17	10.4.17	18 ::00 PM	11.4.17	14::00 PM	124.1	30.9	124.4	140.8	9.3	89.4		19 H	14.5	21.6
86	VELUSWAMY	68	M	POISON	432/17	10.4.17	22 :20 PM	11.4.17	13 .30 PM	123.4	28.4	120.9	134.1	9.2	118.6		15 H30	14.32	17.3
87	NANADABALAN	49	M	POISON	434/17	11.4.17	17 :00 PM	12.4.17	13.30 PM	124.6	31.4	124	138.9	12.4	124.2		20 H 30	22.6	20.3
88	VELMURUGAN	45	M	RTA	436/17	11.4.17	20 :30 PM	12.4.17	14.30 PM	123.9	27.1	123.4	139.4	12.6	123.7		17 H45	23.2	16.7
89	SHANMUGANATHAN	30	F	POISON	439/17	12.4.17	20.15 PM	13.4.17	14 ::00 PM	124.1	31	124.1	141.2	11.8	122.8		18 H45	21.14	20.1
90	SHANMURUGAVALLI	24	F	POISON	467/17	19.4.17	16.15PM	20.4.17	15;00 PM	122.6	31.4	124.4	132.6	12.9	123.5		22H45	23.98	20.33
91	BARNABAI	50	M	POISON	468/17	20.4.17	2:00: AM	20.4.17	15 : 00 PM	121.9	27.6	119.7	133.4	8.9	119.6		13h30	13.66	16.02
92	SUDHAKAR	20	M	POISON	551/17	09.5.17	22:00PM	10.5.17	14 :00 PM	120.6	31.4	119.4	132.6	9.4	121.4		16H25	14.91	20.3
93	MANIKANDAN	22	M	POISON	682/17	2.6.17	23 PM	02.06.17	14.30 PM	119.8	29.7	120.7	134	9.2	122.3		15 H30	14.32	20.3
94	MUTHULAXMI	30	F	RTA	728/17	12.06.17	9.35 AM	12.6.17	15 :35 PM	153	22	106	171	8.2	121	+	6 H	11.6	19.87
95	SRINIVASULU	57	F	RTA	729/17	12.6.17	2.30 AM	12.3.17	15.45 PM	112	30	95.8	132	7.6	87.5	+	13 H30	10.3	18.9
96	AGASTIAN	32	M	POISON	778/17	22.6.17	1:00 AM	22.6.17	11:00 AM	112.8	27.4	118.1	138.5	11.03	101		10H	19.08	16.43
97	VEMBU	35	M	HANGING	779/17	22.6.17	1:00 AM	22.6.17	11.15 AM	112.5	27.2	118.1	146.2	12	123		10 H15	21.66	16.3
98	SUBBAIAH	57	M	RTA	780/17	22.06.17	5:00 AM	22.6.17	11.20 AM	127	30.8	123.8	151	9.7	124		18 H40	15.7	19.7
99	RASAIAH	37	M	SNAKE BITE	857/17	07-09-2017	16.45 PM	10.07.17	12.30 PM	129.6	31.2	126	128.7	12.5	124		19 H45	22.9	20.2
100	RAJU	62	M	RTA	858/17	10.7.17	0.15 AM	10.07.17	12.15 PM	126.6	27.5	117.8	142.1	8.45	116		12 H	12.37	16.44